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Some color in the desert: description of a new species of *Liolaemus* (Iguania: Liolaemidae) from southern Peru, and its conservation status

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Abstract.—The desert of southern Peru and northern Chile is an area with a high degree of endemism in squamate reptiles. In this work, an endemic new species is described in the genus *Liolaemus* with a restricted geographical distribution on the western slopes of the La Caldera batholith in the Department of Arequipa, southern Peru, that inhabits the Desert province of southern Peru, between 1,800 and 2,756 m asl. The new species is characterized by a unique combination of morphological and molecular characters that distinguish it from all other *Liolaemus* species, and it is included in the *L. reichei* clade within the *L. montanus* group. Evidence presented shows that the category of threat corresponds to Endangered under the IUCN Red List criteria.

Keywords. Arequipa, coastal desert, Endangered, La Caldera batholith, *Liolaemus insolitus*, lizard, Reptilia

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Introduction

The Desert province of the South American Transition Zone (*sensu* Morrone 2014), a biogeographic area that corresponds to a narrow strip along the Pacific Ocean coast from northern Peru to northern Chile (Fig. 1), is located in southern Peru near the Chilean border. This desert contains one of the most hyper-arid deserts in the world, the La Joya desert, which includes areas with zero annual rainfall (Valdivia-Silva et al. 2012) and soils with characteristics like the surface of Mars (Valdivia-Silva et al. 2011). The southern portion of the Desert province harbors a distinctive biota characterized by many endemic plants and animals (e.g., Gutiérrez et al. 2019; Málaga et

al. 2020). The knowledge of the amphibians and reptiles in this area remains scarce compared to the desert areas in Chile and Argentina (Escomel 1929; Dixon and Wright 1975; Péfaur et al. 1978a,b; Ceí and Péfaur 1982; Frost 1992; Carrillo and Icochea 1995; Zeballos et al. 2002; Gutiérrez et al. 2010; Abdala y Quinteros 2014); although in recent years three species of *Liolaemus* lizards were described from this region (Aguilar-Puntriano et al. 2019; Villegas-Paredes et al. 2020).

The South American genus *Liolaemus* comprises more than 270 formally described species (Abdala and Quinteros 2014; Gutiérrez et al. 2018; Abdala et al. 2019; Villegas-Paredes et al. 2020; Chaparro et al. 2020). These lizards occupy habitats ranging from hot areas, such as

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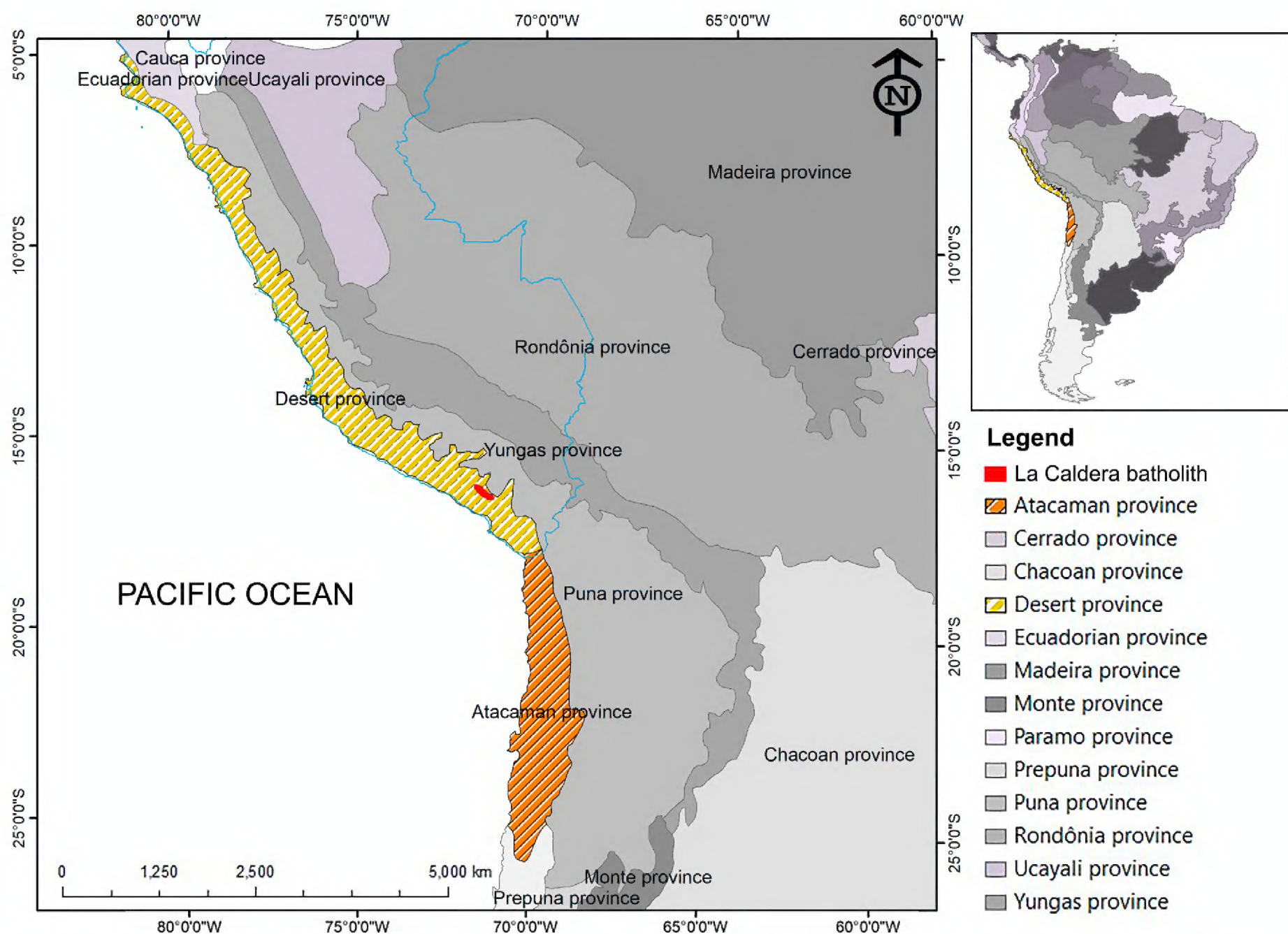


Fig. 1. Biogeographic regionalization proposed by Morrone (2014), showing the limits of the Desert province and Atacama province. The geoform La Caldera batholith, adapted from Ramos (2008), is also shown.

the Atlantic coast of southern Brazil and the continental deserts in Chile, Peru, and Argentina, to very cold regions such as Patagonia in Argentina or the high Central Andes in Peru and Bolivia, and reaching elevations greater than 5,000 m asl (Abdala and Quinteros 2014; Gutiérrez et al. 2018; Abdala et al. 2020; Ruiz et al. 2019; Quinteros et al. 2020).

The great diversity within *Liolaemus* includes a few species with a wide distribution range, such as *L. darwini* (Abdala 2007), *L. multicolor* (Abdala et al. 2020), and *L. wiegmanni* (Villamil et al. 2019), in addition to a large number of species with very restricted distributions, e.g., *L. halonastes* (Lobo et al. 2010), *L. rabinoi* (Abdala et al. 2017), and *L. balagueri* (Villegas-Paredes et al. 2020). *Liolaemus* is divided into the subgenera *Eulaemus* and *Liolaemus sensu stricto* (Laurent 1983, 1985; Schulte et al. 2001). Within these subgenera, a large number of monophyletic groups have been named (Etheridge 1995; Lobo 2005; Avila et al. 2006; Abdala 2007; Quinteros 2013; Breitman et al. 2011; Abdala et al. 2020).

One of the large groups within *Eulaemus* is the *L. montanus* group (Etheridge 1995; Abdala et al. 2020), which is made up of more than 60 described species, and several unnamed species (Abdala et al. 2020). In general, the *L. montanus* group has been studied in recent years from various branches of biology (Halloy et al. 2013;

Troncoso-Yañez 2013; Riveros-Riffo and Torres-Murua 2015; Ruiz de Gamboa and Ortiz-Zapara 2016; Aguilar-Kirigin and Abdala 2016; Aguilar-Kirigin et al. 2016; Quipildor et al. 2018), however the taxonomy (Abdala et al. 2008, 2009, 2013; Lobo et al. 2010; Quinteros and Abdala 2011; Gutiérrez et al. 2018; Ruiz de Gamboa et al. 2018; Aguilar et al. 2017; Aguilar-Puntriano et al. 2019; Abdala et al. 2019), and the phylogenetic hypotheses (Aguilar et al. 2017; Abdala et al. 2020; Chaparro et al. 2020), are the areas that have been most developed, providing essential information for understanding the distribution and diversity of the group. However, essential knowledge gaps remain, including sensitive and important issues such as conservation and natural history. In total, 17 species of *L. montanus* group have been reported for Peru (Chaparro et al. 2020), with six species recorded in the last three years (Gutiérrez et al. 2018; Aguilar-Puntriano et al. 2019; Chaparro et al. 2020; Villegas-Paredes et al. 2020). Additionally, in recent integrative taxonomy studies (Aguilar et al. 2017; Abdala et al. 2020), several populations of unnamed species representing independent lineages have been proposed.

While the *L. montanus* species group largely inhabits cold and high-altitude environments, the species of the *L. reichei* clade (*sensu* Abdala et al. 2020) occupy coastal habitats of northern Chile and southern Peru (e.g., Aguilar-

Table 1. Species list of *Liolaemus reichei* clade.

Species name	Author(s)	Distribution
<i>Liolaemus audituvelatus</i>	(Núñez and Yáñez 1983)	Chile: Antofagasta/ Atacama Regions
<i>Liolaemus balagueri</i>	Villegas et al. 2020	Peru: Arequipa Department
<i>Liolaemus chiribaya</i>	Aguilar et al. 2019	Peru: Moquegua Department
<i>Liolaemus insolitus</i>	Cei y Péfaur 1982	Peru: Arequipa Department
<i>Liolaemus nazca</i>	Aguilar et al. 2019	Peru: Arequipa Department
<i>Liolaemus poconchilensis</i>	Valladares 2004	Peru: Tacna Department, Chile: Arica Region
<i>Liolaemus reichei</i>	(Werner 1907)	Chile: Tarapaca Region
<i>Liolaemus stolzmanni</i>	(Steindachner 1891)	Chile: Antofagasta Region
<i>Liolaemus torresi</i>	(Nuñez et al. 1891)	Chile: Antofagasta Region

Puntriano et al. 2018; Villegas-Paredes et al. 2020). The known diversity of the *L. reichei* clade (Table 1) has increased considerably in recent years with the description of *L. balagueri* (Villegas-Paredes et al. 2020), as well as *L. chiribaya* and *L. nazca* (Aguilar-Puntriano et al. 2019). Various taxonomic and phylogenetic hypotheses have been proposed recently for the *L. reichei* group (Langstroth 2011; Aguilar-Puntriano et al. 2018; Ruiz de Gamboa et al. 2018; Valladares et al. 2018; Abdala et al. 2020; Villegas-Paredes et al. 2020; Chaparro et al. 2020). Abdala et al. (2020) recovered seven candidate species within their *L. reichei* clade which are all very close phylogenetically to *L. insolitus*, a species with a distribution restricted to its type locality in the coastal desert of the Department of Arequipa. In the present study, the taxonomic hypothesis of one of these unnamed populations is evaluated using the general or unified concept of species (De Queiroz 1998, 2007). This concept defines a species as an entity that represents independent historical lineages or divergent lineages of metapopulations. Our criteria to determine the independence of this lineage is based on Total Evidence, such as phylogenetics (molecular and morphological), multivariate statistical analysis, and the description of unique morphological characters; and the results provide decisive evidence to describe it as a new species of *Liolaemus*.

Materials and Methods

Images and maps. Photographs of live specimens were taken using a digital camera Canon sx50 hs. Close-up photographs of the holotype (preserved) were taken with a digital camera Canon EOS Rebel T5. Maps were elaborated using ArcMap 10.3, and use coordinates previously cited by Aguilar et al. (2016), Gutiérrez et al. (2018), and Chaparro et al. (2020). Type localities were taken from the original manuscripts of the species descriptions. Coordinates of the records reported here were obtained with a GPS device (datum WGS84), Garmin Etrex 30. The regionalization map was elaborated using shape files design from Löwenberg-Neto, which follows Morrone (2014).

Material examined. Specimens of *Liolaemus* examined were from the Museo de Historia Natural de la Universidad Nacional de San Agustín de Arequipa, Perú (MUSA); Museo de Biodiversidad del Perú, Cusco, Perú (MUBI); Fundación Miguel Lillo, Tucumán, Argentina (FML); and Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM). Collected specimens of *Liolaemus* were captured by hand within the locality of La Caldera batholith, District of Uchumayo, Province of Arequipa, Department of Arequipa, Peru. Specimens were euthanized with a 1% Halatal solution, fixed with 10% formaldehyde, and stored in 70% alcohol. Prior to fixation, a sample of muscle was collected for DNA extraction and fixed in 96% ethanol. Collected specimens are deposited in the collections of MUSA and MUBI. Appendix I details the specimens used for the first time here, as well as those reanalyzed for the present work but previously examined in Abdala and Quinteros (2008), Abdala et al. (2008, 2009, 2013), Quinteros et al. (2008), Quinteros and Abdala (2011), Gutiérrez et al. (2018), and Abdala et al. (2020). Additional data were obtained from the literature for *L. erroneus* (Núñez and Yáñez 1984), *L. omorfi* (Demangel et al. 2015), and *L. stolzmanni* (Langstroth 2011).

Conservation status and endemism. The IUCN (2001, 2020) criteria were used to categorize the new species. The extent of occurrence (EOO), and area of occupancy (AOO), were obtained using the GeoCat tool (<http://geocat.kew.org/>), which is a tool that follows IUCN criteria. The endemic concept and restricted range of distribution followed Bruchmann and Hobohm (2014), IUCN (2016), Kier and Barthlott (2001), and Noguera-Urbano (2017).

Morphological data. Morphological characters utilized in taxonomic studies of *Liolaemus* were studied here, mainly those described or cited by Laurent (1985), Etheridge (1995, 2000), Abdala (2007), Abdala and Juárez (2013), Gutiérrez et al. (2018), Aguilar-Puntriano et al. (2018), Villegas-Paredes et al. (2020), and Abdala et al. (2020). The coloration description was based on live specimens and digital photographs taken in the field. Color

pattern terminology follows Lobo and Espinoza (1999), Abdala (2007), and Abdala et al. (2020). Examination of scalation or pholidosis was performed using a binocular stereoscope (10–40x), and morphometric measurements were made with a Mitutoyo caliper with precision of 0.01 mm. The morphometric variables were measured three times on the same individual, and the mean value for each species was used in the statistical analyses. Only adult males were used in the multivariate analysis to avoid confounding effects of intraspecific allometric variation, and to avoid confusion in the multivariate analyses due to possible sexual dimorphism (Losos 1990; Abdala et al. 2019). All bilateral characters were measured on the right side. The measured morphometric traits and meristic characters counted follow Abdala et al. (2019) [Appendix II].

DNA extraction, amplification, and sequencing.

Total genomic DNA was extracted from samples of muscle using the GenElute mammalian genomic DNA miniprep kit (Sigma-Aldrich), according to the manufacture's instructions. A fragment of approximately 1,174 base pairs of the mitochondrial gene cytochrome b (*cyt-b*) was amplified by polymerase chain reaction (PCR), using the primers IguaCytob_F2 (5'-CCACCGTTGTTATTCAACTAC-3') and IguaCytob_R2 (5'-GGTTTACAAGACCAATGCTTT-3') [Corl et al. 2010]. Each reaction contained 1x PCR buffer (KCl), 2.5 mM MgCl₂, 0.25 mM each dNTP, 0.1 μM each primer, 1 unit of Taq DNA polymerase (Thermo Scientific), and 1 μL DNA extract. PCR cycling consisted of a 5 min initial denaturation at 94 °C, 35 cycles of 30 sec at 94 °C; 30 sec at 55 °C; 60 sec at 72 °C, and a final elongation step of 2 min at 72 °C. The PCR product was visualized on 1.5% agarose gel stained with Gel-Red (Biotium, Inc.), and subsequently sent to Macrogen, Inc. (Seoul, Republic of Korea) for purification and direct sequencing. The nucleotide sequence was visualized and edited using 4 Peaks software (<http://nucleobytes.com/4peaks/>) and checked manually, and nucleotides with ambiguous positions were clarified. The sequences newly obtained in this study are publically available in GenBank (see Table 2).

Statistical analysis. A Principal Component Analysis (PCA) was employed to analyze morphological variation, and discriminant function analyses (DFA) were used to verify morphological variation between and within each *Liolaemus* species employing a jackknife classification matrix (Manly 2000; McCune and Grace 2002; Quinn and Keough 2002; Zar 2010). Based on the existing phylogenetic results (Abdala et al. 2020) and those obtained, four species of *L. reichei* clade distributed in Peru (*L. balagueri*, *L. chiribaya*, *L. insolitus*, and *L. nazca*), and the new species proposed here were used as comparative groups for building the PCA and the DFA. Normal distributions of the morphometric data were

examined using the Kolmogorov-Smirnov test ($P < 0.05$), and homoscedasticity was evaluated with Levene's test. To reduce the effect of non-normal distributions of the morphological data, all continuous variables were log₁₀ transformed and meristic variables were square root transformed (Irschick and Losos 1996; Sokal and Rohlf 1998; Peres-Neto and Jackson 2001).

All operational taxonomic units were analyzed by two distinct treatments. The PCA analysis was performed to evaluate the distribution of individuals corresponding to the five species (*L. balagueri*, *L. chiribaya*, *L. insolitus*, *L. nazca*, and *Liolaemus* sp. nov.) in the multivariate space. The PCA was based on the correlation matrices of the morphological variables to reduce dimensionality of the data (Quinn and Keough 2002; Lovett et al. 2000). The PCA and DFA were evaluated separately for continuous and meristic characters, following the recommendations of certain authors not to join both matrices in the multivariate analyses, although there is no mathematical consensus on this approach (McGarigal et al. 2000). The PCA evaluates relationships within a single group of interdependent variables regardless of any relationships that they may have outside of that group of variables. After the PCA was performed, and the lineal combinations that explained the highest variation were extracted, DFA was performed independently for continuous and meristic morphological characters, to identify the combination of morphological characters that best differ between the groups identified by the PCA. The DFA produces a linear combination of variables that maximizes the probability of correctly assigning observations to predetermined groups, and simultaneously, new observations can be classified into one of the groups, providing likelihood values of such classification (McGarigal et al. 2000; Van den Brink et al. 2003). All statistical analyses were performed using Statistica software, version 7.0 (<http://www.statsoft.com>).

Phylogenetic analysis. Three matrices were constructed, including: (1) morphological data; (2) molecular characters (*cyt-b*); and (3) both morphological and molecular data. Total Evidence and morphological phylogenetic analysis were performed using the matrix of Abdala et al. (2020). The morphological matrix includes 306 characters and 105 terminals (with *Ctenoblepharys adspersa* and *Phymaturus palluma* as an “outgroup” and 96 terminals of *L. montanus* group). The Total Evidence matrix included 105 terminals and 3,390 characters. Parsimony was used as the optimality criterion, only selecting the shortest trees or those with the fewest homoplasies. TNT version 1.5 (Tree Analysis Using New Technology; Goloboff et al. 2003) was employed to generate the phylogenetic hypotheses. Continuous characters were analyzed following Goloboff et al. (2006), and were standardized using the function *mkstandb.run*. For this analysis, the value of two was considered as the highest transformation cost. Heuristic searching was used to find the shortest trees or those with the smallest number of steps. The matrix was analyzed

Table 2. GenBank codes and voucher information of *Liolaemus* and outgroup specimens sequenced for this study.

Species names	Voucher code	cyt- <i>b</i>	Source
<i>Ctenoblepharys adspersa</i> (outgroup)	BYU 50502	MH981364	Aguilar-Puntriano et al. 2018
<i>L. annectens</i>	BYU 50489	KX826616	Aguilar et al. 2016
<i>L. annectens</i>	BYU 50486	KX826615	Aguilar et al. 2016
<i>L. annectens</i>	BYU 50491	KX826617	Aguilar et al. 2016
<i>L. annectens</i> “Lampa”	MUSM 31433	KX826618	Aguilar et al. 2016
<i>L. balagueri</i>	MUSA 5575	MK568539	Villegas-Paredes et al. 2020
<i>L. balagueri</i>	MUSA 5576	MK568538	Villegas-Paredes et al. 2020
<i>L. chiribaya</i>	BYU 51568	MH981365	Aguilar-Puntriano et al. 2018
<i>L. etheridgei</i>	BYU 50494	KX826620	Aguilar et al. 2016
<i>L. etheridgei</i>	BYU 50495	KX826621	Aguilar et al. 2016
<i>L. etheridgei</i>	BYU 50497	KX826622	Aguilar et al. 2016
<i>L. etheridgei</i>	BYU 50493	KX826619	Aguilar et al. 2016
<i>L. etheridgei</i>	BYU 50499	KX826623	Aguilar et al. 2016
<i>L. etheridgei</i>	MUSM 31494	KX826625	Aguilar et al. 2016
<i>L. stolzmanni</i>	LNC 138	MH184793	Ruiz De Gamboa et al. 2018
<i>L. stolzmanni</i>	MR 213	MH184794	Ruiz De Gamboa et al. 2018
<i>L. torresi</i>	LNC 146	MH184797	Ruiz De Gamboa et al. 2018
<i>L. torresi</i>	LNC 134	MH184795	Ruiz De Gamboa et al. 2018
<i>L. torresi</i>	LNC 133	MH184796	Ruiz De Gamboa et al. 2018
<i>L. insolitus</i>	MUSM 31490	KX826627	Aguilar et al. 2016
<i>L. insolitus</i>	BYU 50462	KX826626	Aguilar et al. 2016
<i>L. dorbignyi</i>	LJAMMCNP 5002	KF968848	Olave et al. 2014
<i>L. eleodori</i>	LJAMMCNP 2709	KF968850	Olave et al. 2014
<i>L. audituvelatus</i>	LNC 136	MH184785	Ruiz De Gamboa et al. 2018
<i>L. audituvelatus</i>	LNC 86	MH184779	Ruiz De Gamboa et al. 2018
<i>L. audituvelatus</i>	ER1	MH184780	Ruiz De Gamboa et al. 2018
<i>L. audituvelatus</i>	MUAP104	MH184782	Ruiz De Gamboa et al. 2018
<i>L. audituvelatus</i>	SSUC-Re760	MH184783	Ruiz De Gamboa et al. 2018
<i>L. audituvelatus</i>	LNC 135	MH184784	Ruiz De Gamboa et al. 2018
<i>L. vallecurensis</i>	LJAMMCNP 650	KF968960	Olave et al. 2014
<i>L. nazca</i> (<i>L.</i> “Nazca”)	BYU 50472	KX826673	Aguilar et al. 2016
<i>L. nazca</i> (<i>L.</i> “Nazca”)	BYU 50507	KX826674	Aguilar et al. 2016
<i>L. nazca</i> (<i>L.</i> “Nazca”)	BYU 50508	KX826675	Aguilar et al. 2016
<i>L. nazca</i> (<i>L.</i> “Nazca”)	MUSM 31523	KX826676	Aguilar et al. 2016
<i>L. nazca</i> (<i>L.</i> “Nazca”)	MUSM 31524	KX826677	Aguilar et al. 2016
<i>L. ortizi</i>	MUSM 31513	KX826633	Aguilar et al. 2016
<i>L. ortizi</i>	MUSM 31514	KX826634	Aguilar et al. 2016
<i>L. aff. poconchilensis</i>	MUSM 31545	KX826637	Aguilar et al. 2016
<i>L. poconchilensis</i>	MUSM 31543	KX826635	Aguilar et al. 2016
<i>L. poconchilensis</i>	MUSM 31544	KX826636	Aguilar et al. 2016
<i>L. poconchilensis</i>	MZUC43498	MH184798	Ruiz De Gamboa et al. 2018
<i>L. poconchilensis</i>	MZUC43497	MH184799	Ruiz De Gamboa et al. 2018

Table 2 (continued). GenBank codes and voucher information of *Liolaemus* and outgroup specimens sequenced for this study.

Species names	Voucher code	cyt- <i>b</i>	Source
<i>L. polystictus</i>	MUSM 31451	KX826642	Aguilar et al. 2016
<i>L. polystictus</i>	MUSM 31446	KX826641	Aguilar et al. 2016
<i>L. qalaywa</i>	MUBI 12081	MT366061	Chaparro et al. 2020
<i>L. qalaywa</i>	MUBI 12099	MT366062	Chaparro et al. 2020
<i>L. “Apurimac”</i>	MUSM 27694	MH981371	Aguilar-Puntriano et al. 2018
<i>L. robustus</i>	MUSM 31504	KX826646	Aguilar et al. 2016
<i>L. robustus</i>	MUSM 31508	KX826648	Aguilar et al. 2016
<i>L. robustus</i>	MUSM 31505	KX826647	Aguilar et al. 2016
<i>L. robustus</i>	BYU 50483	KX826643	Aguilar et al. 2016
<i>L. thomasi</i>	BYU 50469	KX826680	Aguilar et al. 2016
<i>L. thomasi</i>	BYU 50466	KX826678	Aguilar et al. 2016
<i>L. thomasi</i>	MUSM 31516	KX826681	Aguilar et al. 2016
<i>L. thomasi</i>	BYU 50467	KX826679	Aguilar et al. 2016
<i>L. thomasi</i>	MUBI 5925	MT366060	Chaparro et al. 2020
<i>L. signifer</i>	MUSM 31443	KX826656	Aguilar et al. 2016
<i>L. signifer</i>	MUSM 31434	KX826654	Aguilar et al. 2016
<i>L. signifer</i>	BYU 50444	KX826652	Aguilar et al. 2016
<i>L. signifer</i>	BYU 50357	KX826651	Aguilar et al. 2016
<i>L. signifer</i>	BYU 50350	KX826649	Aguilar et al. 2016
<i>L. signifer</i>	MUSM 31437	KX826655	Aguilar et al. 2016
<i>L. signifer</i>	BYU 50355	KX826650	Aguilar et al. 2016
<i>L. signifer</i>	MUSM 31447	KX826657	Aguilar et al. 2016
<i>L. signifer</i>	MUSM 29110	KX826653	Aguilar et al. 2016
<i>L. melanogaster</i>	BYU 50151	KX826628	Aguilar et al. 2016
<i>L. melanogaster</i>	MUSM 31472	KX826630	Aguilar et al. 2016
<i>L. melanogaster</i>	MUSM 31475	KX826631	Aguilar et al. 2016
<i>L. melanogaster</i>	BYU 50154	KX826629	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31371	KX826665	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31374	KX826667	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31373	KX826666	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	BYU 50426	KX826661	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31461	KX826668	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	BYU 50430	KX826663	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31462	KX826669	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	BYU 50431	KX826664	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	BYU 50428	KX826662	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31464	KX826670	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31465	KX826671	Aguilar et al. 2016
<i>L. victormoralesii</i> (<i>L. “AbraToccto”</i>)	MUSM 31468	KX826672	Aguilar et al. 2016
<i>L. williamsi</i>	BYU 50463	KX826684	Aguilar et al. 2016
<i>L. williamsi</i>	MUSM 31485	KX826687	Aguilar et al. 2016
<i>L. williamsi</i>	BYU 50143	KX826682	Aguilar et al. 2016

Table 2 (continued). GenBank codes and voucher information of *Liolaemus* and outgroup specimens sequenced for this study.

Species names	Voucher code	cyt- <i>b</i>	Source
<i>L. williamsi</i>	BYU 50464	KX826685	Aguilar et al. 2016
<i>L. williamsi</i>	BYU 50144	KX826683	Aguilar et al. 2016
<i>L. williamsi</i>	MUSM 31486	KX826688	Aguilar et al. 2016
<i>L. williamsi</i>	BYU 50465	KX826686	Aguilar et al. 2016
<i>L. “AbraApacheta”</i>	MUSM 31481	KX826660	Aguilar et al. 2016
<i>L. “AbraApacheta”</i>	BYU 50145	KX826658	Aguilar et al. 2016
<i>L. “AbraApacheta”</i>	BYU 50148	KX826659	Aguilar et al. 2016
<i>L. polystictus</i> “Castrovirreyna”	MUSM 31454	KX826639	Aguilar et al. 2016
<i>L. polystictus</i> “Castrovirreyna”	BYU 50630	KX826638	Aguilar et al. 2016
<i>L. polystictus</i> “Castrovirreyna”	BYU 31455	KX826640	Aguilar et al. 2016
<i>L. robustus</i> “MinaMartha”	BYU 50438	KX826644	Aguilar et al. 2016
<i>L. robustus</i> “MinaMartha”	MUSM 31439	KX826645	Aguilar et al. 2016
<i>L. annectens</i>	LDHV 73	MT773391	This study
<i>L. aff. annectens</i>	LECG 078	MT773392	This study
<i>L. “Cotahuasi”</i>	RGP 6031	MT773393	This study
<i>L. “Cotahuasi”</i>	MDUM 006	MT773394	This study
<i>L. “Cotahuasi”</i>	MDUM 005	MT773395	This study
<i>L. “Cotahuasi”</i>	MDUM 004	MT773396	This study
<i>L. aff. qalaywa</i> 1	MDUM 001	MT773397	This study
<i>L. aff. qalaywa</i> 1	MDUM 002	MT773398	This study
<i>L. aff. qalaywa</i>	MDUM 017	MT773399	This study
<i>L. aff. qalaywa</i>	MDUM 014	MT773400	This study
<i>L. aff. qalaywa</i>	MDUM 007	MT773401	This study
<i>L. aff. qalaywa</i>	VOI 009	MT773402	This study
<i>L. aff. qalaywa</i>	VOI 006	MT773403	This study
<i>L. chiribaya</i>	AQR 003	MT773404	This study
<i>L. chiribaya</i>	AQR 004	MT773405	This study
<i>L. aff. insolitus</i> 4	RGP 6249	MT773406	This study
<i>L. sp. nov.</i> (described herein)	MUSA 1766	MT773407	This study
<i>L. sp. nov.</i> (described herein)	MUBI 13522	MT773408	This study
<i>L. sp. nov.</i> (described herein)	MUBI 14417	MT773409	This study
<i>L. aff. insolitus</i> 6	MUSA 1769	MT773410	This study
<i>L. aff. insolitus</i> 6	MUSA 1770	MT773411	This study
<i>L. aff. insolitus</i> 6	MUSA 1771	MT773412	This study
<i>L. insolitus</i>	AQR 001	MT773413	This study
<i>L. insolitus</i>	AQR 002	MT773414	This study
<i>L. aff. balagueri</i>	LDHV 005	MT771288	This study
<i>L. aff. insolitus</i> 2	RGP 6147	MT773415	This study
<i>L. aff. insolitus</i> 8	RGP 6154	MT773416	This study

using the “implied weights” method (Goloboff 1993). The value of the constants $K = 14$ (morphological analysis) and $K = 19$ (Total Evidence analysis) were used as in the analysis of Abdala et al. (2020). One thousand replications

were performed for each search. Symmetric resampling was used to obtain support values for the results obtained, with 500 replications with a deletion probability of 0.33. To construct the cyt-*b* tree, sequences from this study

(13 species) were combined with a published dataset of 24 species, and five undescribed lineages of *Liolaemus* (Aguilar et al. 2016; Aguilar-Puntriano et al. 2018, 2019; Chaparro et al. 2020; De Gamboa et al. 2018; Olave et al. 2014; Villegas-Paredes et al. 2020) [Table 2]. A maximum likelihood phylogenetic analysis was carried out with MEGA X (Kumar et al. 2018). Heuristic tree searches were performed with the GTR + G + I substitution model (determined based on the Akaike information criterion), and 1,000 bootstrap replications.

Results and Discussion

The independent taxonomic status of the population of *Liolaemus* studied here was validated using morphological and molecular evidence. The results of the phylogenetic and statistical analyses described below suggest that the population can be considered as distinctive from all other described species of *Liolaemus*. In accordance with best practices in zoological nomenclature, the results of statistical, morphological, and molecular phylogenetic analyses are provided following the formal presentation of the new proposed species.

Taxonomy

Liolaemus anqapuka Huamaní-Valderrama, Quiroz, Gutiérrez, Aguilar-Kirigin, Chaparro, Abdala **sp. nov.** (Figs. 2–5).

urn:lsid:zoobank.org:act:EF6ABFF4-97BC-4C8F-83E7-79D2B3FE7171

1885 *Ctenoblepharis adpersus*—Boulenger, *Catalogue of the Lizards in the British Museum (Natural History). Second Edition* 2: 136–137.

1978b “*Ctenoblepharus* sp.” Péfaur et al. *Bulletin de l'Institut Français d'Études Andines* VII (1–2): 129–139.

1982 *Liolaemus insolitus* Ceí and Péfaur, In *Actas 8vo Congreso Latinoamericano de Zoología*. Pp. 573–686.

1995 *Ctenoblepharys adpersa*—Etheridge, *American Museum Novitates* 3142: 1–34.

2004 *Phrynosaura* [sp.] Nuñez, *Noticiario Mensual Museo de Historia Natural* 353: 28–34.

2010 *Liolaemus* cf. *insolitus*, Gutierrez and Quiroz, *Herpetofauna del Sur del Perú*, Available: <http://herpetofaunadelsurdelperu.blogspot.com> [Accessed: 13 June 2020].

2011 *Liolaemus* species 2, Langstroth, *Zootaxa* 2809: 32.

2020 *Liolaemus* aff. *insolitus*7, Abdala et al., *Zoological Journal of the Linnean Society* 189: 1–29.

Holotype. MUSA 5573, an adult male (Figs. 2–3), from between Quebrada San Jose and Quebrada Tinajones, District of Uchumayo, Province of Arequipa, Department of Arequipa, Peru (16°31'47"S, 71°39'04"W) at 2,460 m asl, collected on 10 November 2013, by C.S. Abdala, R. Gutiérrez, A. Quiroz, L. Huamani, and J. Cerdeña.

Paratypes. Six adult females: MUSA 5574–75, same data as holotype. MUSA 1766, from Quebrada Tinajones, 300 m southeast of holotype (16°31'54.29"S, 71°38'57.547"W) at 2,492 m asl, collected on 9 October 2010, by A. Quiroz and J. Cerdeña. MUBI 13522, MUSA 1767, from Quebrada Tinajones, 600 m southeast of holotype (16°31'54.207"S, 71°38'46.187"W) at 2,528 m asl, collected on 9 October 2010, by A. Quiroz and J. Cerdeña. MUBI 14680, from Quebrada Tinajones (16°31'22.705"S, 71°37'35.666"W) at 2,561 m asl, collected on 27 July 2007, by R. Gutiérrez and A. Quiroz. **Two adult males:** MUBI 13521, from Quebrada Tinajones, 300 m southeast of holotype (16°31'54.29"S, 71°38'57.547"W) at 2,492 m asl, collected on 9 October 2010, by A. Quiroz and J. Cerdeña. MUBI 14417, from Quebrada Tinajones (16°31'22.705"S, 71°37'35.666"W) at 2,561 m asl, collected on 27 July 2007, by R. Gutiérrez and A. Quiroz.

Diagnosis. We assign *Liolaemus anqapuka* **sp. nov.** to the *L. montanus* group because it presents a blade-like process on the tibia, associated with the hypertrophy of the tibial muscle *tibialis anterior* (Abdala et al. 2020; Etheridge 1995) and its placement in the morphological and molecular phylogenies (Fig. 11). Within the *L. montanus* group, *Liolaemus anqapuka* **sp. nov.** differs from *L. andinus*, *L. annectens*, *L. aymararum*, *L. cazianiae*, *L. chlorostictus*, *L. dorbignyi*, *L. fabiani*, *L. forsteri*, *L. foxi*, *L. graciellae*, *L. huayra*, *L. inti*, *L. jamesi*, *L. melanogaster*, *L. multicolor*, *L. nigriceps*, *L. orientalis*, *L. pachecoi*, *L. pantherinus*, *L. patriciaturrae*, *L. pleopholis*, *L. polystictus*, *L. puritamensis*, *L. qalaywa*, *L. robustus*, *L. scrocchii*, *L. signifer*, *L. vallecurensis*, *L. victormoralesii*, *L. vulcanus*, and *L. williamsi*, for being species of larger size (SVL greater than 75 mm) unlike *L. anqapuka* **sp. nov.**, which has a maximum SVL of 73.5 mm. *Liolaemus anqapuka* **sp. nov.**, has between 58 and 72 (mean = 64.8) scales around the body, which differentiates it from species of the group with more than 80 scales, such as *L. cazianiae*, *L. duellmani*, *L. eleodori*, *L. erguetae*, *L. forsteri*, *L. graciellae*, *L. molinai*, *L. multicolor*, *L. nigriceps*, *L. patriciaturrae*, *L. pleopholis*, *L. poecilochromus*, *L. porosus*, *L. pulcherrimus*, *L. robertoi*, *L. rosenmanni*, *L. ruibali*, and *L. vallecurensis*; and also from species with less than 55 scales, like *L. aymararum*, *L. jamesi*, *L. pachecoi*, and *L. thomasi*. *Liolaemus anqapuka* **sp. nov.** have 60–72 dorsal scales (mean = 65.5), and differs from *L. andinus*, *L. cazianiae*, *L. eleodori*, *L. erguetae*, *L. forsteri*, *L. foxi*, *L. graciellae*, *L. halonastes*, *L. molinai*, *L. multicolor*, *L. nigriceps*, *L. patriciaturrae*, *L. pleopholis*, *L. poecilochromus*, *L. porosus*, *L. pulcherrimus*, *L. robertoi*, *L. rosenmanni*, *L. ruibali*, *L. schmidtii*, and *L. vallecurensis*, which have between 75–102 dorsal scales. The number of ventral scales between 73–87 (mean = 81.3) differentiates it from species with more than 90 ventral scales, such as *L. andinus*, *L. cazianiae*, *L. erguetae*, *L. eleodori*, *L. foxi*, *L.*

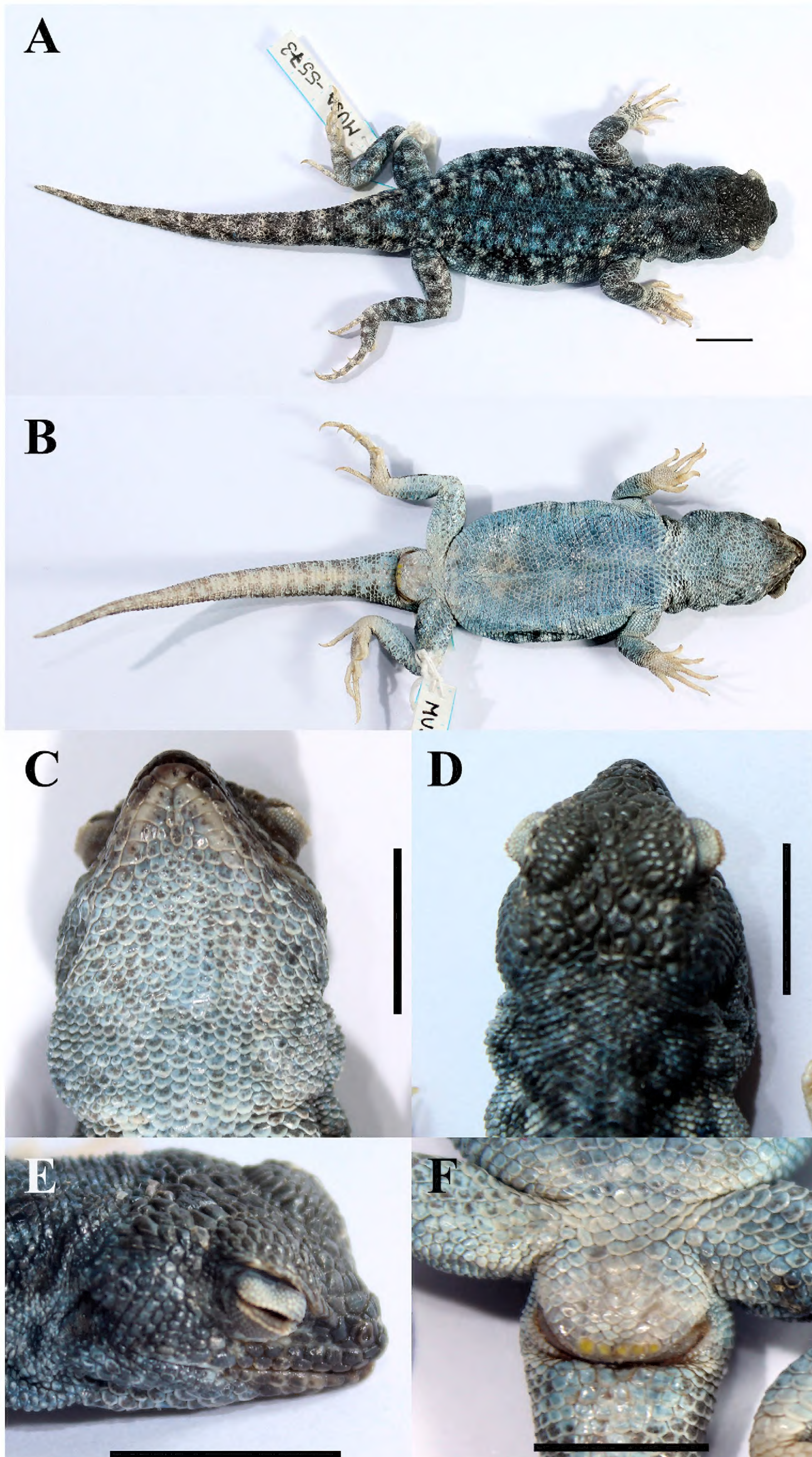


Fig. 2. Details of the holotype of *Liolaemus anqapuka* sp. nov. (MUSA 5573; SVL = 73.5 mm, Tail = 63.9 mm): (A) dorsal and (B) ventral views of body; (C) ventral, (D) dorsal, and (E) lateral views of head; (F) ventral view of precloacal pores. Scale = 10 mm.



Fig. 3. Adult male of the holotype, *Liolaemus anqapuka* **sp. nov.** (MUSA 5573; SVL = 73.5 mm, Tail = 63.9 mm), from the Department of Arequipa, 2,460 m asl. Photos by C.S. Abdala.

gracielae, *L. halonastes*, *L. hajeki*, *L. molinai*, *L. nigriceps*, *L. patriciaturrae*, *L. pleopholis*, *L. poecilochromus*, *L. porosus*, *L. robertoi*, *L. rosenmanni*, and *L. vallecurensis*. *Liolaemus anqapuka* **sp. nov.** has juxtaposed or subimbricate dorsal scales, without keel or mucron, this differentiates it from species with conspicuous keel and mucron, as *L. aymararum*, *L. etheridgei*, *L. famatinae*, *L. fittkaui*, *L. griseus*, *L. huacahuasicus*, *L. montanus*, *L. orko*, *L. ortizi*, *L. polystictus*, *L. pulcherrimus*, *L. qalaywa*, *L. signifer*, *L. tajzara*, *L. thomasi*, *L. victormoralesii*, and *L. williamsi*. Females of *L. anqapuka* **sp. nov.** present 1–4 (mean = 2.6) precloacal pores, this character differentiates it from species like *L. andinus*, *L. balagueri*, *L. fittkaui*, *L. multicolor*, *L. ortizi*, *L. polystictus*, *L. puritamensis*, *L. robertoi*, *L. robustus*, *L. rosenmanni*, *L. ruibali*, *L. thomasi*, and *L. vallecurensis*, because they do not present precloacal pores in females.

Liolaemus anqapuka **sp. nov.** belongs to the clade of *Liolaemus reichei* *sensu* Abdala et al. (2020). The color pattern of *Liolaemus anqapuka* **sp. nov.** has a combination of characteristics in males and females that distinguish it from the rest of the *Liolaemus* of the group. The number of scales around the body is between 58–72 (mean = 64.8), which differentiates it from *L. audituvelatus*, *L. balagueri*, *L. insolitus*, and *L. reichei* (Table 3). The number of dorsal scales varies between 60–72 (mean = 65.5), which is lower than the number in *L. audituvelatus*, higher than in *L. nazca*, and has a variation in range of scales different than *L. chiribaya*, *L. reichei*, and *L. torresi* (Table 3). The numbers of ventral scales of *Liolaemus anqapuka* **sp.**

nov. vary between 73–87 (mean = 81) which are different from *L. audituvelatus*, *L. nazca*, and *L. torresi* (Table 3). The presence of precloacal pores in females 1–4 (mean = 2.6), is different from *L. audituvelatus*, *L. balagueri*, and *L. reichei*, whose females do not have precloacal pores (Table 3). Coloration patterns on lateral sides have light blue scales, which are different from *L. audituvelatus*, *L. balagueri*, *L. nazca*, *L. torresi*, and *L. reichei* (Table 3). The existence of dorsal body scales with a keel differentiate it from *L. nazca* which have dorsal body scales without keel. Ventral thigh scales with keel are present in 100% of individuals of *L. anqapuka* **sp. nov.** but they are less evident than those present in *L. chiribaya*, where only 35% of individuals present this character (Table 3). The maximum SVL is greater than in *L. audituvelatus*, *L. poconchilensis*, *L. reichei*, *L. stolzmanni*, and *L. torresi* (Table 3).

Description of the holotype (Figs. 2–3). Adult male (MUSA 5573), SVL 73.53 mm. Head 1.20 times greater in length (16.47 mm) than width (13.74 mm). Head height 10.48 mm. Neck width 14.37 mm. Eye diameter 3.67 mm. Interorbital distance 10.96 mm. Orbit-auditory meatus distance 6.55 mm. Auditory meatus 2.0 mm high, 0.97 mm wide. Orbit-commissure of mouth distance 5.77 mm. Internasal width 1.58 mm. Subocular scale length 4.09 mm. Trunk length 31.81 mm, width 24.37 mm. Tail length 63.91 mm. Femur length 14.65 mm, tibia 14.47 mm, and foot 18.01 mm. Humerus length 11.01 mm. Forearm length 9.31 mm. Hand length 10.82 mm. Pygal



Fig. 4. Male specimens of the *Liolaemus anqapuka* sp. nov. Photos by A. Quiroz (A–D) and C.S. Abdala (E).

region length 5.95 mm, and cloacal region width 7.97 mm. Dorsal surface of head rough, with 17 scales, rostral 3.09 times longer (2.78 mm) than wide (0.9 mm). Mental as long (2.78 mm) as rostral, trapezoidal, surrounded by four scales. Nasal separated from rostral by one scale. Two internasals slightly longer than wide. Nasal surrounded by eight scales, separated from canthal by two scales. Nine scales between frontal and rostral. Frontals divided into three scales. Interparietal smaller than parietal, in contact with six scales. Preocular separated from lorilabials by one scale. Five superciliaries and 15 upper ciliaries scales. Three differential scales at anterior margin of auditory

meatus. Ten temporary scales. Four lorilabials scales, in contact with subocular. Seven supralabials, which are not in contact with subocular. Five supraocular. Eight lorilabials. Six infralabials. Five chin shields, 4th pair separated by five scales. Seventy scales around half a body.

Sixty-two rounded dorsal body scales, juxtaposed, and without a keel or mucron; laminar anterior on members, imbricate and slightly keeled; laminar on hind limbs, imbricate and slightly keeled; tail with dorsal scales in the first third juxtaposed, and the remaining two-thirds imbricate, presence of some scales keeled. Eighty-six



Fig. 5. Female specimens of the *Liolaemus anqapuka* sp. nov. Photos by A. Quiroz.

ventral scales, from the mental to the cloacal region, following the ventral midline of the body, laminar, imbricated. Thirty-two imbricate gulars, smooth. Neck with longitudinal fold with 36 granular, not keeled scales, ear fold and antehumeral fold present. Gular fold incomplete. Forelimbs ventrally laminar, subimbricate to imbricate, not keeled; hind legs laminar, imbricate, with some keeled scales (Figs. 2–3). Seventeen subdigital lamellae on the 4th finger of the hand. Twenty-one subdigital lamellae of the 4th toe, with four keels, plantar scales with keels and mucrons. Lamellar ventral scales on tail, imbricate, not keeled. Five precloacal pores. Supernumerary pores absent.

Color of holotype in life (Fig. 3). Dorsal and lateral color of the neck is light gray with few light blue scales, with dull orange scales, and spots on side. Dorsum, limbs, and tail light gray. Vertebral region delimited, vertebral line and spots absent, but dotted with sky blue scales. Paravertebral and dorsolateral region of the body, large orange spots of irregular shape and size stand out. These orange spots are surrounded and dotted with numerous sky-blue scales, with thin design or undulating edges. The orange spots with light white irregular spots. There are no dorsolateral bands, antehumeral arch, or scapular spots. On the sides of the body the pattern of orange spots and

light blue scales is repeated, but the gray color of the body is darker. This design extends to the first third of the tail. Tail with dark semi-complete rings with white back spots. Midline of the body with orange scales and spots. Back of the limbs with numerous light white spots unevenly distributed. Hands and feet dorsally white. Ventrally white from mental region to the tail. Gular and femoral regions light yellow. Flanks of the body with a thin orange border from the armpits to the groin.

Morphological variation. Twenty-two specimens (six males and 16 females). Dorsal surface of head rough with 14–21 scales (mean = 16.82; STD = 1.71). Nasal surrounded by 6–9 scales (mean = 7.41; STD = 0.73). Supralabials 7–10 scales (mean = 8.18; STD = 0.8), lorilabials 8–11 scales (mean = 9.32; STD = 0.89). A line of lorilabial scales. Supraoculars 4–6 (mean = 5.45; STD = 0.6). Interparietals smaller than parietals, surrounded by 4–8 scales (mean = 6.32; STD = 1.09). Infralabials 6–9 (mean = 7.14; STD = 0.77). Gulars 28–39 (mean = 33.41; STD = 2.99). Temporals smooth, 7–10 scales (mean = 9.09; STD = 0.97). Meatus auditory higher 1.37–2.47 mm (mean = 2.05; STD = 0.26), than wide 0.20–1.20 (mean = 0.81; STD = 0.25). Head longer 12.32–17.20 (mean = 14.91; STD = 1.31) than wide 9.15–15.92 (mean = 12.77; STD = 2.03). Head height 6.84–10.48 (mean = 8.38; STD

Table 3. Differences in morphological characters of the *Liolaemus reichei* clade.

Morphological character	<i>L. auditvetulus</i> (n = 4)	<i>L. balagueri</i> (n = 18)	<i>L. reichei</i> (n = 3)	<i>L. insolitus</i> (n = 10)	<i>L. poconchilensis</i> (n = 4)	<i>L. torresi</i> (n = 8)	<i>L. chiribaya</i> (n = 10)	<i>L. nazca</i> (n = 7)	<i>L. angapuka</i> sp. nov. (n = 22)
Snout-vent length	47.2 (47.5) 47.8	50.9 (59.1) 66.5	41.5 (47.7) 50.8	50.3 (59.4) 65.6	47.17 (51.7) 53.83	53.8 (58.1) 64	49.28 (59.60) 68.25	53.51 (59.35) 64.34	52.15 (63.92) 73.53
Tail length	45.3 (46.3) 47.5	48.7 (59.4) 80.0	35.7 (39.5) 43.1	45.4 (51.7) 61.9	42.66 (47.2) 53.65	58.8 (57.4) 74	46.34 (56.47) 68.89	60.26 (70.55) 79.60	46.77 (56.83) 67.16
Snout-vent length/ Tail length	0.95 (0.98) 1.01	0.93 (1.02) 1.09	-	0.82 (0.91) 0.98	-	-	0.89 (0.98) 1.07	-	0.76 (0.90) 1.05
Head length	11.4 (11.6) 11.7	12.4 (14.1) 17.1	10 (10.9) 11.5	11.6 (13.6) 14.9	11.34 (13.1) 14.16	13 (13.6) 14.5	12.18 (14.00) 15.63	12.82 (14.35) 16.46	12.32 (14.90) 17.2
Head width	9.6 (9.8) 10.3	9.8 (11.2) 13.6	8.3 (8.5) 9.7	9.5 (11.5) 12.4	9.51 (10.9) 11.53	10.3 (11.0) 11.7	8.92 (10.29) 11.44	8.99 (10.12) 11.74	9.15 (12.76) 15.92
Forelimb length	27.1 (28.6) 28.7	21.0 (22.9) 25.1	21.4 (22.6) 24.4	26.1 (29.1) 31.5	22.2 (24.8) 27.5	26.3 (28.8) 30.5	25.17 (28.41) 29.87	19.22 (23.10) 25.91	25.68 (28.71) 31.53
Hind limb length	37.1 (38.8) 40.6	29.1 (32.4) 35.9	30.1 (31.7) 33.4	37.4 (41.2) 44.3	31.15 (34.1) 32.62	38.6 (40.1) 42.2	36.13 (39.79) 42.68	32.05 (35.06) 37.92	36.02 (41.28) 47.13
Lorilabials scales	7 (8) 9	5 (6.9) 8	8 (8.7) 9	7 (8.2) 10	14	8 (8.4) 9	5 (5.5) 6	7 (8.4) 10	8 (9.32) 11
Auditory meatus scales	0	1	1	1 (1.5) 3	1 (2) 3	1	1	1 (1.86) 2	1 (1.55) 3
Upper ear scale	0 (1) 2	0	0	1	0 (0.75) 1	0	0 (0.85) 1	1	1
Diagonal large atrial scale	0 0.5) 1	0	1	1	1	1	1	1	1
Neck scales	45 (45) 45	29 (31) 38	26 (28.7) 33	35 (36.7) 38	35 (36.5) 38	44 (45.9) 47	28 (28.4) 32	28 (30.86) 32	32 (38.86) 43
Scales around midbody	74 (74.5) 75	52 (54.0) 56	43 (45) 47	51 (55.4) 58	57 (59.4) 62	64 (66.6) 72	55 (61.8) 66	53 (56) 60	58 (65.09) 72
Dorsal scales	78 (82.5) 87	50 (55.1) 68	50 (51.7) 54	56 (62.9) 68	57 (61.2) 64	70 (74.4) 80	52 (57.4) 61	53 (54.14) 56	60 (65.59) 72
Ventral scales	86 (90.5) 95	65 (73.4) 79	80 (86.4) 88	70 (73.9) 78	77 (79.6) 84	86 (90.6) 97	67 (72.7) 77	65 (70.57) 74	73 (81.32) 87
Dorsal scale arrangement of the body	Juxtaposed and sub-juxtaposed	Sub-juxtaposed	Juxtaposed	Sub-juxtaposed and Imbricated	Juxtaposed and imbricated	Juxtaposed	Juxtaposed and sub-juxtaposed	Imbricated	Juxtaposed
Keel scales	absent	absent	absent	absent	absent	absent	absent	present	absent
Percentage of individuals with ventral keel scales in the center of the thigh	0%	0%	0%	0%	0%	0%	35%	0%	100%

Table 3 (continued). Differences in morphological characters of the *Liolaemus reichei* clade.

Morphological character	<i>L. audivetulus</i>	<i>L. balagueri</i>	<i>L. reichei</i>	<i>L. insolitus</i>	<i>L. poconchilensis</i>	<i>L. torresi</i>	<i>L. chiribaya</i>	<i>L. nazca</i>	<i>L. anqapuka</i> sp. nov.
Arrangement of celestial scales in males	absent	absent	absent	Regular, from the occiput to approximately two thirds of the tail	Both sides of the body	absent	Regular, associated with paravertebral and lateral	absent	Light blue scales that can form thin irregular lines or clump together forming conspicuous spots
Arrangement of celestial scales in females	absent	absent	absent	Scales, next to paravertebral spots	absent	absent	absent	absent	Sometimes light blue lateral scales on paravertebral spots
Green side spots	absent	present	absent	absent	absent	absent	absent	present	absent
Precloacal pores in females	0	0	0	0 (1.3) 3	0 (1.3) 2	0 (1.5) 2	2 (3) 4	1 (2) 3	3 (4.22) 5
Precloacal pores in males	5	3 (5.08) 7	4	5 (6.2) 7	4 (4.6) 5	3	3 (4.14) 6	3 (4) 6	4 (5) 6

= 0.87). Underarm to groin length 21.61–32.8 (mean = 28.58; STD = 2.76). SVL males 56.23–73.53 mm (mean = 65.05 mm; STD = 7.08) and females 52.15–71.10 mm (mean = 62.9 mm; STD = 4.61). Femur length 10.11–14.65 mm (mean = 12.31 mm; STD = 1.06). Humerus length 7.56–11.01 mm (mean = 8.86 mm; STD = 0.99). Forearm length 7.65–11.56 mm (mean = 9.59 mm; STD = 1.06). Hand length 8.03–11.25 (mean = 10.25; STD = 0.86). Scales around midbody 58–72 (mean = 65.09; STD = 3.7). Dorsal 60–72 (mean = 65.59; STD = 3.5), juxtaposed to sub-juxtaposed, and smooth scales. Infradigital lamellae of the 4th finger of the hand 15–21 (mean = 17.73; STD = 1.45) and of the 4th toe 20–26 (mean = 21.67; STD = 1.5). Ventral 73–87 (mean = 81.32; STD = 3.37) larger than dorsal scales. Tail length 46.77–67.16 mm (*n* = 17, mean = 56.83 mm; STD = 5.91). Males with 4–6 (mean = 4.67; STD = 0.82) precloacal pores, and females with 3–5 (mean = 4.22; STD = 0.83) precloacal pores. Body measurements, males (mean = 66.62 mm) slightly larger than females (mean = 62.90 mm), tail length in males slightly larger (mean = 61.74 mm) than females (mean = 54.80 mm) [Table 4].

Color variation in life (Figs. 4–5). *Liolaemus anqapuka* sp. nov. shows evident sexual dichromatism. In males, head is darker than the gray body. In some specimens, supralabial and infralabial scales are generally lighter gray than the rest of the head. The subocular is generally white with irregular dark spots. The dorsal color of the neck is gray, varying in its hue, and may be dotted with some light blue scales and orange spots. The body color is always gray. The vertebral region in most males is well delimited with some light blue scales. No vertebral line, dorsolateral bands, antehumeral arch, or scapular spots. Few specimens have diffuse gray paravertebral spots, and rounded shape. As in the holotype, in the paravertebral, dorsolateral, and lateral regions of the body, irregular orange spots stand out, surrounded and dotted with celestial scales. Orange spots can vary in intensity and size, as light blue scales that can form thin irregular lines or clump together to form more conspicuous spots. In some specimens the amount of light blue scales is so remarkable that they cover the orange spots. Orange spots and light blue scales are distributed on the sides of the tail. In some individuals, the celestial scales reach the distal end of the tail. In some specimens, light blue scales are replaced by dark, bluish-green scales. In some, irregularly shaped white spots are distributed among the orange spots. The fore and hind limbs, as well as the tail, have the same design as the body. In the tail, incomplete rings of dark spots with light edges are formed. Ventrally, the majority of males are similar. The predominant color is white, some have faint yellow and a yellow hue that can vary in intensity, highlighted in the gular region and the hind limbs. On the sides of the belly, a thin orange longitudinal line protrudes from the armpit to the groin (Fig. 4).

Table 4. Differences in morphological characters between males and females of *Liolaemus anqapuka* sp. nov.

Morphological characters	Mean in males	STD males	Variation in males	Mean in females	STD females	Variation in females
Snout-vent length	66.62	6.05	(56.23–73.53)	62.91	4.61	(52.15–71.10)
Tail length	61.74	3.74	(58.08–67.16)	54.78	5.49	(46.77–66.88)
Head length	15.9	0.85	(14.87–17.2)	14.53	1.27	(12.32–16.79)
Head width	13.94	1.46	(11.5–15.92)	12.33	2.08	(9.15–15.38)
Forelimb length	30.45	0.77	(29.41–31.53)	28.05	1.58	(25.68–31.36)
Hind limb length	44.03	2.33	(39.99–47.13)	40.25	2.9	(36.02–45.04)
Head length/snout-vent length	0.24	0.02	(0.22–0.26)	0.23	0.01	(0.21–0.25)
Head length/head width	1.15	0.09	(1.04–1.29)	1.2	0.13	(1.02–1.37)
Trunk width/trunk length	0.7	0.06	(0.64–0.78)	0.69	0.1	(0.53–0.97)
Tympanum height/tympanum width	2.74	1.07	(2.06–4.9)	3.08	2.21	(1.57–10.7)
Auditory meatus scales	1.5	0.55	(1–2)	1.56	0.63	(1–3)
Neck scales	39.33	3.5	(34–42)	38.7	3.91	(32–43)
Scales around midbody	65.67	4.59	(60–72)	64.9	3.46	(58–72)
Dorsal scales	67.17	4.58	(61–72)	65	2.97	(60–72)
Ventral scales	83.5	2.51	(81–87)	80.5	3.35	(73–84)
Pygal scales	6.5	2.07	(5–10)	6.75	1.69	(5–10)
Precloacal pores	4.67	0.82	(4–6)	3.64	1.15	(2–5)

Females have a totally different coloring pattern than males (Fig. 5). The color of the head varies from brown to gray, with some dark red spots and scales. The supralabial, infralabial, and lorilabial scales are lighter in color than the dorsal surface of the head. The back of the body can be light gray or brown; with small paravertebral spots, gray or dark brown, and circular or sub-quadrangular; with a small white spot on the back which can be the same size as the paravertebral; and with meager orange spots between the paravertebrals. A few females have light blue scales on paravertebral spots. On the sides of the body, there may be lateral spots of the same design as the paravertebral ones. The tail and hind limbs have the same design and color as the body, without dorsolateral bands. Ventrally they are white or faint yellow immaculate throughout the body. In some females, the tail has more intense yellow throughout its extension (Fig. 5).

Etymology. The specific name refers to the coloration patterns of males. The word “anqapuka” is an original word in the Quechua language (spoken currently in the Peruvian Andes), corresponding to a complex word between “anqa” assigned to the blue color, and “puka” which means orange or red color.

Distribution and natural history. *Liolaemus anqapuka* sp. nov. is restricted to the western slopes of the La Caldera batholith, Arequipa, Peru, between 1,800 and 2,756 m asl, which includes the upper altitude limit of the La Joya desert (Fig. 6). The distribution is within the Desert biogeographic province (*sensu* Morrone 2014). *Liolaemus anqapuka* sp. nov. inhabits arid

environments, characteristic of the desert of southern Peru, with sandy-stony substrates and little slope, seasonal herbaceous vegetation, and columnar and prostrate cacti. This species also inhabits sectors without vegetation (Fig. 7). It takes refuge mainly under stones, and in burrows that surround the roots of small bushes, prostrate cacti, and in cavities underground or in hardened sand. Some specimens of *Liolaemus anqapuka* sp. nov. were observed feeding on coleopteran larvae, as well as larvae and notably adults of Lepidoptera belonging to the Sphingidae family (Fig. 8). Feeding on beetles is very similar to that reported for the closely-related species *Liolaemus insolitus*, which is specialized in feeding on so-called “flea beetles” of the subfamily Halticinae (Coleoptera: Chrysomelidae) [Cei and Péfaur 1982]. The adults and larvae of the family Sphingidae are most abundant in the summer months, when the local rainfall is complemented by abundant ephemeral surface watercourses whose flow is derived from rainfall on the western slopes of the Andes, and these insects can display unusual and explosive development. During years when there is exceptionally high accumulated rainfall, a biological phenomenon known as a “blooming desert” can occur (Chavez et al. 2019), and some phytophagous insects would be expected to be able to use the abundant plant resources that suddenly become available in these events, as reported for Sphingidae in northern Chile (Vargas and Hundsdoerfer 2019). *Liolaemus anqapuka* sp. nov. was found in syntopy with other reptile species, such as *Microlophus* sp. and *Phyllodactylus gerrhopygus*.

Endemism, threats, and conservation status. *Liolaemus anqapuka* sp. nov. is considered as an endemic species

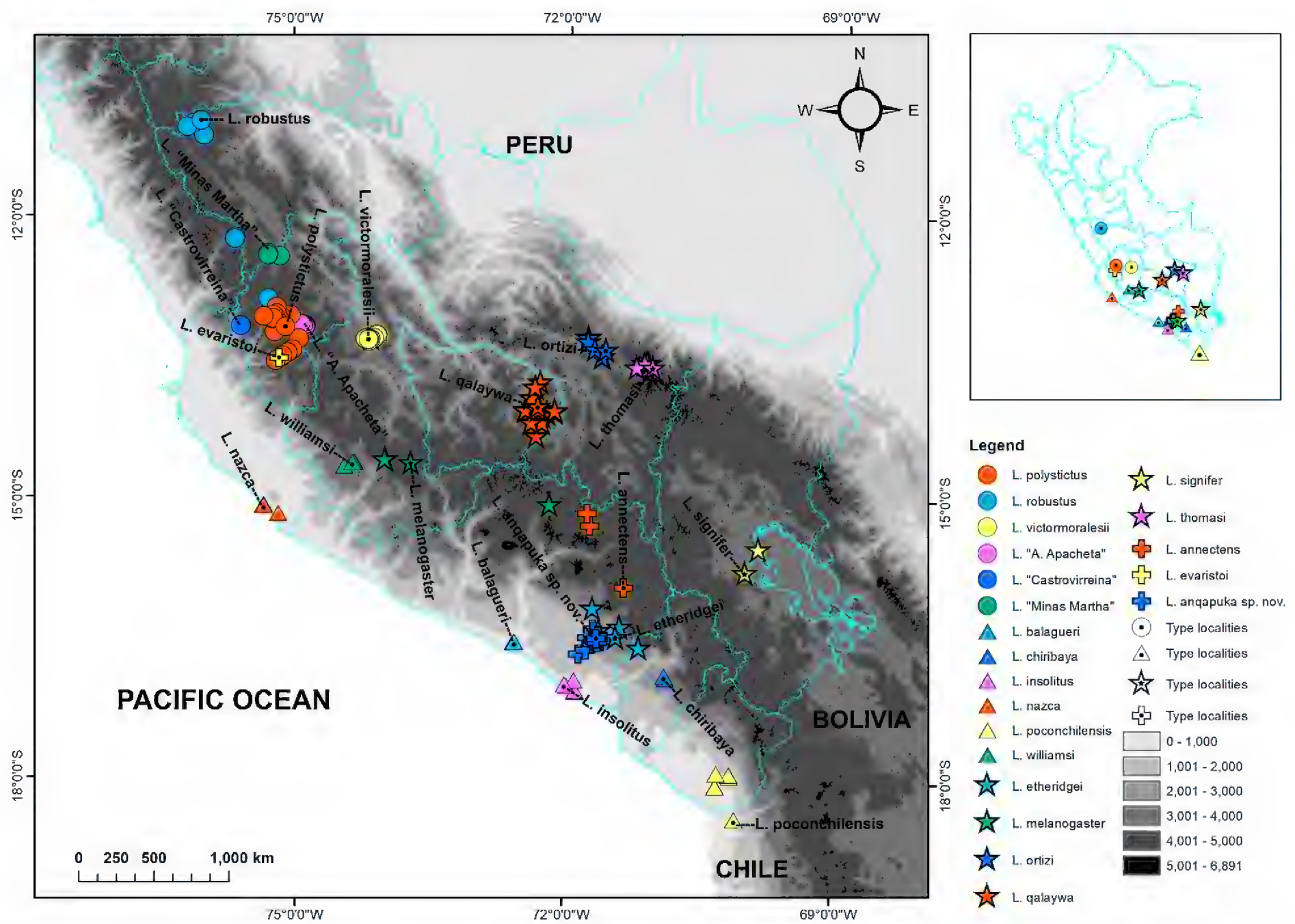


Fig. 6. Geographic distribution of *Liolaemus montanus* group species from Peru. Symbols with a black dot in the middle represent the type locality of each species. Species with quotation marks in the names belong to the candidate species listed in Aguilar et al. (2016).

with a restricted-range of geographical distribution, because the species occupancy is less than 10,000 km² (Bruchmann and Hobohm 2014; IUCN 2016; Kier and Barthlott 2001; Noguera-Urbano 2017). Using the Geocat tool, and based on records of the species, we estimate the extent of occurrence (EOO) at 147.2 km² and the area of occupancy (AOO) at 80.0 km². The restricted range might be caused by their climatic tolerance, and the ecological adaptation to extreme environmental conditions found on the Desert biogeographic province. The main threats are the loss of habitat, because of the large-scale mining activities, urban expansion, and contamination by chemicals and metals; and also because of the presence of highways that cut through their natural habitat, and the opening of new secondary roads. Following the IUCN (2020) criteria, and using the actual knowledge of the new species, we evaluated the conservation status of *L. anqapuka* **sp. nov.** to be in the category of endangered EN [A2cde; A3cde; A4cde] [B1ab (i, iii) + 2abc (ii, iii, iv)], based on the area of occupancy (AOO) < 500 km², the extent of occurrence (EOO) < 5,000 km², the number of localities are ≤ 5; and we consider it as a species with restricted range because *L. anqapuka* **sp. nov.** has a global range size less than or equal to 10,000 km² (IUCN 2016).

Statistical analysis (Figs. 9–10). The summary statistics for all the non-transformed, continuous, and meristic characters taken from five species of *Liolaemus* are shown in Appendix II. The homogeneity of variance was not supported for either continuous or meristic characters by the Levene's test in some groups. Therefore, the results of the Principal Component Analyses (PCA) should be preferred for deriving linear combinations of the variables that summarize the variation in the data set. The results of the PCA for continuous and meristic characters are presented separately (Tables 5–6).

The first four components of continuous characters explained 55.51% of the variation, and a screen plot test of the PCs indicated that only the first three components contained nontrivial information. The first axis represents body size, loading negatively for most variables, and accounts for 23.46% of the variation, with strong loading for width of the base of the tail. The second axis represents morphological variation and accounts for most of the remaining variation, with strong loadings for mental scale width, length of the 4th supralabial scale, and upper width of the pygal area. The next axes account for the remaining variation.

The first four components of meristic characters explained 54.59% of the variation, and a screen plot

Table 5. Principal component (PC) axes loadings of continuous characters for *L. balagueri* ($n = 12$), *L. chiribaya* ($n = 10$), *L. insolitus* ($n = 15$), *L. nazca* ($n = 7$), and *Liolaemus anqapuka* **sp. nov.** ($n = 7$). Eigenvectors, eigenvalues, and percentage of variance explained for the first four principal components from transformed data in the five putative species of *Liolaemus*.

Loadings	PC1	PC2	PC3	PC4
Percentage variation accounted for	23.46	14.84	10.97	6.24
Eigenvalue	7.27	4.6	3.4	1.93
Snout-vent length	−0.85	−0.06	0.09	0.16
Minimum distance between the nasal scales	−0.13	0.48	0.67	−0.02
Snout width at the edge of the flake canthal	−0.04	0.2	0.54	0.2
Distance from the nose to the back edge of the flake canthal	−0.68	−0.08	−0.15	0.08
Distance between the posterior edge of the series superciliary	−0.67	0.56	0.01	0.23
Length of the interparietal	−0.48	0.08	−0.44	−0.29
Length of the parietal	−0.51	0.43	−0.20	−0.27
Mental flake width	0.13	0.73	0.49	0.05
Length of the mental scale	−0.50	−0.33	−0.68	−0.16
Distance from nostril to the mouth	−0.55	−0.43	0.28	0.01
Rostral height	−0.51	−0.19	0.16	0.05
Length of the subocular scale	−0.41	−0.19	0.01	0.06
Ear height	−0.16	−0.23	0.22	−0.49
Ear width	0.11	0.29	0.67	−0.32
Length of the preocular scales	−0.11	−0.56	0.19	0.14
Preocular width	−0.26	−0.46	0.32	0
Length of the fourth supralabial flake	−0.25	−0.71	0.17	−0.17
Length of the fourth lorilabial flake	−0.50	−0.46	0.04	0.04
Length between orbits	−0.61	0.37	−0.05	0.46
Length of the first finger of the forelimb, without the claw	−0.54	0.41	−0.16	−0.29
Length of the claw of the fourth finger of the forelimb	−0.15	0.32	−0.56	0.29
Length of the fifth finger of the forelimb, without the claw	−0.19	0.17	0.23	−0.68
Humerus width	−0.62	0.06	−0.03	0.24
Distance from the insertion of the forelimb in the body toward the elbow	−0.67	0.17	0.29	0.12
Thigh width	−0.66	−0.50	−0.01	−0.23
Length of the first finger of the hind limb, without the claw	−0.24	0.35	−0.21	−0.38
Length of the claw of the fourth finger of the hind limb	−0.54	0.19	−0.15	−0.26
Body width	−0.62	−0.12	0.53	−0.02
Width of the base of the tail	−0.75	−0.12	0.22	0.19
Upper width of the pygal area	−0.19	0.7	−0.11	−0.13
Length of the pygal area	−0.62	0.4	−0.17	0.01

test of the PCs indicated that only those components contain relevant information. The four axes represent morphological variation, loading strongly for number of paravertebral spots in the right side, number of scales around midbody, number of ventral scales, and number of gular scales. The four axes account for the remaining variation, albeit with values below 0.70 for subdigital lamellae of the 4th finger of the forelimb, number of auricular scales, projecting scales on anterior edge of auditory meatus, and number of organs in the postrostral scales.

The positions of species based on their scores for the two morphological principal components axes are illustrated

in Figs. 9–10. The spatial distribution of the continuous characters indicates that they are sufficient to virtually separate the five Peruvian *Liolaemus* species of the *L. reichei* group. These species can also be distinguished by their position in the analysis of meristic characters only. In both analyses, *Liolaemus anqapuka* **sp. nov.** can be differentiated from other phylogenetically related species by its body size and morphological variation.

To further clarify the position of the *Liolaemus* species in the morphospace of both continuous and meristic characters, a DFA was carried out, where the group membership was determined *a priori*. The result obtained through the DFA for the five species of *Liolaemus* was

Table 6. Principal component (PC) axes loadings of meristic characters for *L. balagueri* ($n = 12$), *L. chiribaya* ($n = 10$), *L. insolitus* ($n = 15$), *L. nazca* ($n = 7$), and *Liolaemus anqapuka* **sp. nov.** ($n = 7$). Eigenvectors, eigenvalues, and percentage of variance explained for the first four principal components from transformed data in the putative species of *Liolaemus*.

Loadings	PC1	PC2	PC3	PC4
Percentage variation accounted for	26.62	10.3	9.63	8.04
Eigenvalue	8.78	3.4	3.18	2.65
Number of scales around the interparietal scale	−0.06	−0.36	−0.03	0.05
Supralabials number on the right side	−0.04	−0.52	−0.27	0.18
Supralabials number on the left side	0.17	−0.51	−0.47	0.42
Infralabials number on the right side	0.39	−0.30	−0.44	−0.01
Infralabials number on the left side	0.25	−0.55	−0.47	−0.07
Number of scales around mental scale	0.37	−0.09	0	−0.11
Number of scales around the rostral scale	0.56	0.31	−0.26	−0.40
Number of lorilabials	−0.16	−0.56	0.07	−0.45
Hellmich index	0.32	−0.10	−0.39	0.4
Subdigital lamellae of the first finger of the forelimb	−0.09	−0.59	0.48	−0.04
Subdigital lamellae of the second finger of the forelimb	0.06	−0.35	0.47	0.44
Subdigital lamellae of the third finger of the forelimb	−0.31	−0.07	0.55	0.2
Subdigital lamellae of the fourth finger of the forelimb	−0.74	−0.12	−0.14	0.24
Subdigital lamellae of the fifth finger of the forelimb	−0.61	0.12	0.38	−0.22
Subdigital lamellae of the first toe of the hind limb	−0.43	−0.37	0.04	0.14
Subdigital lamellae of the second toe of the hind limb	−0.56	−0.40	0.46	−0.16
Subdigital lamellae of the third toe of the hind limb	−0.47	−0.26	0.14	−0.13
Subdigital lamellae of the fourth toe of the hind limb	−0.08	−0.55	0.23	−0.48
Subdigital lamellae of the fifth toe of the hind limb	−0.19	0.22	0.19	0.52
Number of dorsal scales between the occiput and the level of the anterior edge of the thigh	0.43	−0.51	−0.40	−0.18
Precloacal number of pores	0.29	−0.24	0.11	0.5
Number of scales between canthal and nasal	−0.60	−0.41	−0.15	0.36
Number of scales around the nasal scale	−0.20	−0.12	−0.05	−0.09
Supraoculars number enlarged scale in the right side	0.67	−0.22	0.2	−0.27
Supraoculars number enlarged scale in the left side	0.48	−0.23	0.05	−0.48
Number of scales between canthal and nasal scales	0.7	−0.26	0.15	−0.09
Number of organs in the third lorilabial scale	−0.08	−0.18	0.58	0.2
Number of organs above the row of lorilabials scales and below the canthal and preocular scales	0.66	0.02	0.34	−0.13
Gular number of scales	−0.88	0.01	−0.27	−0.25
Number of scales around the middle body	−0.92	0	−0.27	−0.09
Number of ventral scales	−0.92	0.03	−0.26	−0.15
Number of auricular scales	−0.73	0.04	−0.02	−0.31
Number of paravertebral spots in the right side	−0.93	−0.02	−0.23	−0.10

not significant for continuous morphological characters (Wilk’s Lambda = 0.85, $F = 0.71$, $P = 0.60$), and the jackknife classification was 100% satisfactory. The DFA of operational taxonomic units for meristic characters was not significant either (Wilk’s Lambda = 0.69, $F = 1.58$, $P = 0.23$); however, the jackknife satisfactory classification was developed at a 100% rate. These results show *L. anqapuka* **sp. nov.** can be reliably distinguished from

the other species by a combination of morphological characters.

Phylogenetic analysis (Fig. 11). The objective of the phylogenetic analyses carried out (morphological, molecular, and Total Evidence) is not to resolve the relationships of the *L. montanus* group, which is far beyond the scope of this study. The main objective of

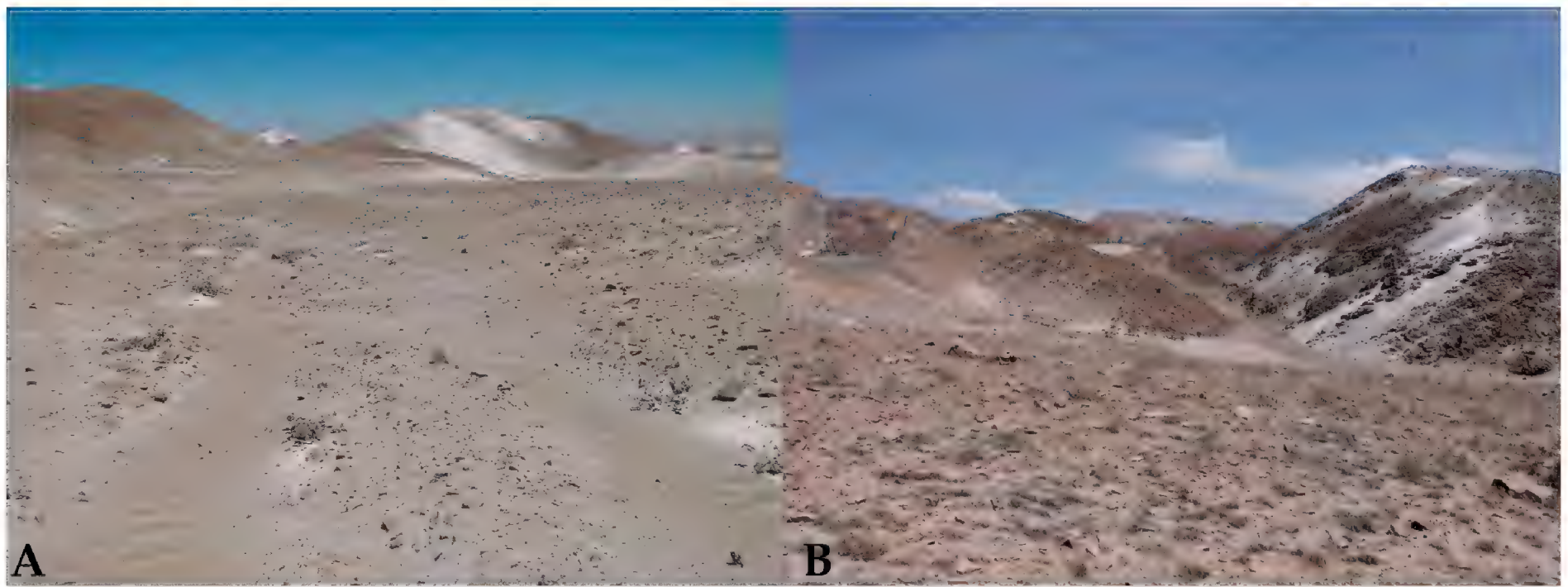


Fig. 7. Habitat of *Liolaemus anqapuka* **sp. nov.** in (A) dry season and (B) wet season. Photos by A. Quiroz (A), C.S. Abdala (B).

these analyses is to obtain some approximation of the phylogenetic relationships of *L. anqapuka* **sp. nov.** and the rest of the *L. reichei* group *sensu* Abdala et al. (2020). The new taxon was recovered in three analyses, within the *L. montanus* group. In the morphological and Total Evidence analyses, under parsimony methodology, the *L. reichei* group is monophyletic; within this, *L. anqapuka* **sp. nov.**, through molecular analysis of ML, the *L. reichei* group is paraphyletic.

Molecular analysis. The three DNA (cyt-*b*) obtained for *L. anqapuka* **sp. nov.** fall within the same clade, supporting the identification of the new species. The nearest terminal is *L. aff. insolitus*4, a population innominate from Department of Arequipa, and it is grouped in the same clade with *L. chiribaya*, a species from Department of Moquegua, with node support (BS = 99). The clade that contains these three species is deeply separated from its sister clade, (*L. poconchilensis* + *L. aff. insolitus*8). The analysis does not recover the clade of *L. reichei* group *sensu* Abdala et al. (2020) as monophyletic.

Morphological analysis. The result of the morphological phylogenetic hypothesis shows that *Liolaemus anqapuka* **sp. nov.** belongs to the group of *L. montanus*, within the clade of *L. reichei* *sensu* Abdala et al. (2020), together with *L. audituvelatus*, *L. balagueri*, *L. chiribaya*, *L. insolitus*, *L. nazca*, *L. poconchilensis*, *L. reichei*, *L. torresi*, and eight unnamed populations so far. *Liolaemus reichei* *sensu* Abdala et al. (2020), is supported by 13 synapomorphies, of which four are continuous characters (lower number of scales from rostral to occiput, lower number of scales around midbody and lower ratio of tail length/SVL) and eight are discrete (ventral scales of the body equal to, or slightly larger than the dorsal; sides of the body not conspicuously colored, with little or no ventral sexual dichromatism; absence of white line in the temporal region; diameter of the eye, larger than the distance between the anterior margin of the eye, and the rostral scale; isognathic profile, substrate where they occur predominantly sandy).

This clade is divided into two large subclades, one with unnamed species and populations from Chile (*L. audituvelatus*, *L. poconchilensis*, *L. reichei*, and *L. torresi*) and the other with species and populations from central and southern Peru (*L. balagueri*, *L. chiribaya*, *L. insolitus*, and *L. nazca*). This last subkey is where the new species is recovered, supported by 19 synapomorphies, several of which stand out: ratio of auditory meatus height/head height, number of pygals, number of lorilabials contacting the subocular, number of supraoculars, dorsal surface of head (rugose), scales on external edge of forelimbs (subimbricate), scales of dorsal hind limbs (subimbricate), with notch in edge of scales of gular fold, scales of pygal region (subimbricate), with dark line through the eye; white posterior edge of paravertebral spots in both sex (present), black dots scattered on dorsal region of hind limbs in males (absent), and dark line through the eye in females (present). *Liolaemus anqapuka* **sp. nov.** have populations of close relatives which also occur in Department of Arequipa, Peru, with particular morphological characteristics, and these are currently under description. *Liolaemus anqapuka* **sp. nov.** is recovered as a sister species of *L. aff. insolitus*4, a population related to *L. insolitus* near the



Fig. 8. *Liolaemus anqapuka* **sp. nov.** eating a moth of the Sphingidae family. Photo by A. Quiroz.

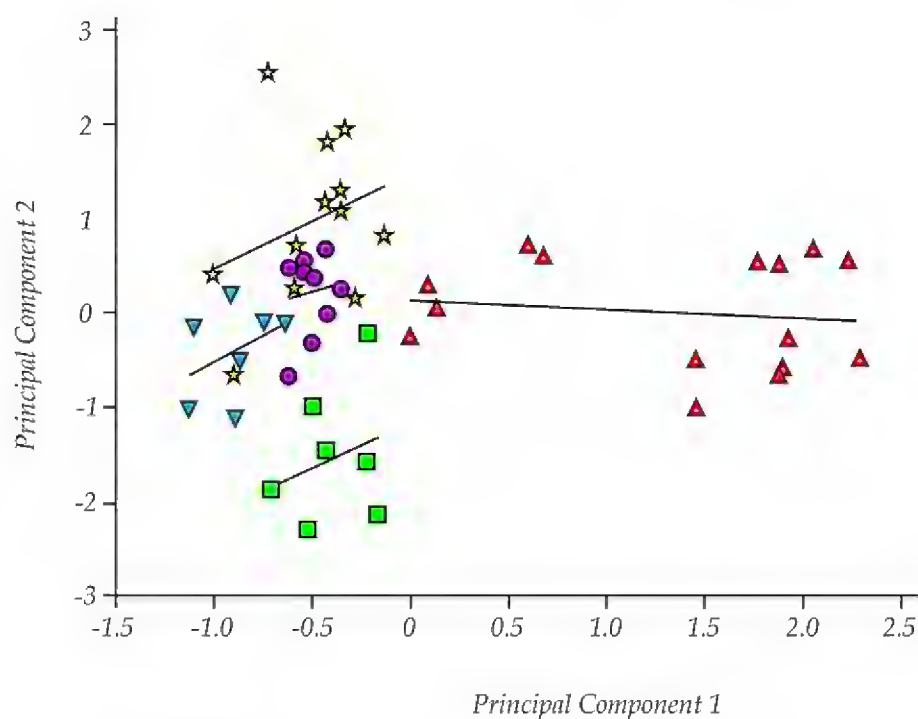


Fig. 9. Plot of principal component scores for continuous characters for *L. balagueri* (yellow stars, $n = 12$), *L. chiribaya* (purple circles, $n = 10$), *L. insolitus* (red triangles, $n = 15$), *L. nazca* (sky blue triangle, $n = 7$), and *L. anqapuka* **sp. nov.** (green squares, $n = 7$). Eigenvectors, eigenvalues, and percent of variation explained for the first two principal components are summarized in Table 5.

coasts of the Department of Arequipa, which occupies elevations of 1,000 m asl. This relationship is supported by six synapomorphies. *Liolaemus anqapuka* **sp. nov.** is supported by seven autapomorphies in the phylogenetic tree (Fig. 11).

Total Evidence analysis (Fig. 11). The *L. reichei* clade is recovered as monophyletic, and *L. anqapuka* **sp. nov.** belongs to this clade, as do the sister species of *L. aff. insolitus*4, as well as in the morphological and molecular phylogenetics analyses. This relationship is supported by 14 synapomorphies, six of which are continuous characters and the support of this relationship is high (89%). This relationship is recovered within the clade (*L. aff. insolitus*5 (*L. aff. insolitus*4 + *L. anqapuka* **sp. nov.**)), and is supported by three morphological and 11 molecular synapomorphies. Likewise, a total of seven autapomorphies support the new species of *Liolaemus*. In this hypothesis, as in the morphological one, two sub clades are recovered within the *L. reichei* clade—on the one hand are the species that are distributed in northern Chile, and on the other are those in southern Peru.

Taxonomic history. Boulenger (1885) identified a male specimen (BMNH 65–5–3–3) from “Arequiba, 7,500 ft” as *Ctenoblepharis adpersus* (an unjustified emendation of *Ctenoblepharys adpersa* Tschudi 1845) in his catalogue of the lizards in the British museum. Péfaur et al. (1978b) mentioned the distribution and classification of the reptiles from Department of Arequipa, noting that the specimens collected by Duellman (1974) from the “La Caldera batholith” located approximately 10 km southwest of Uchumayo town would be “*Ctenoblepharus* sp.” (= *Ctenoblepharys*). But this was not the only mistake. Years later, Cei and Péfaur (1982) wrote the

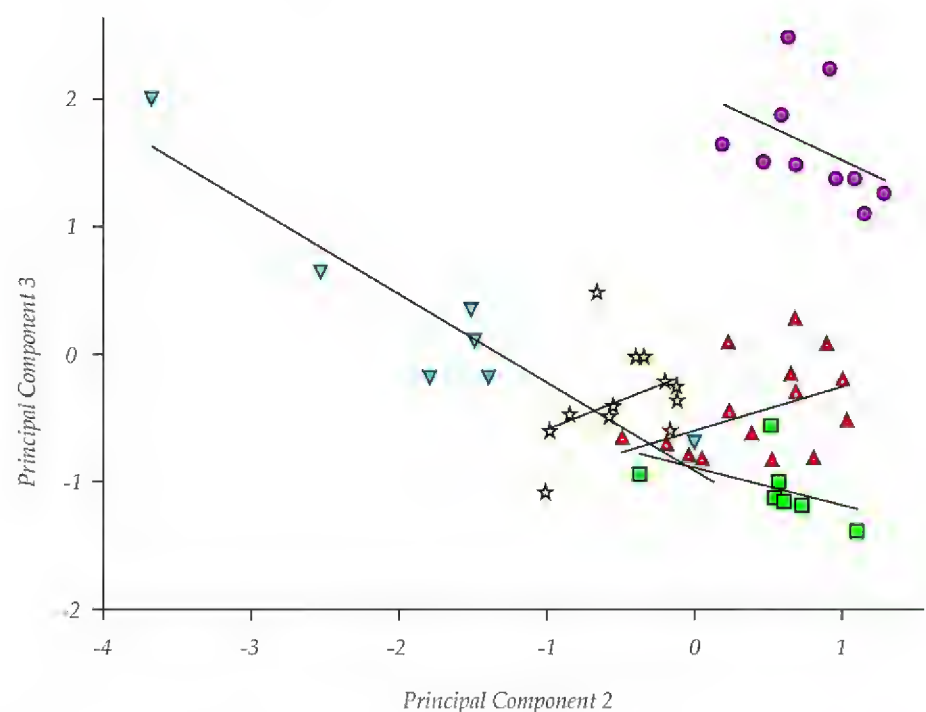


Fig. 10. Plot of principal component scores for meristic characters for *L. balagueri* (yellow stars, $n = 12$), *L. chiribaya* (purple circles, $n = 10$), *L. insolitus* (red triangles, $n = 15$), *L. nazca* (sky blue triangle, $n = 7$), and *L. anqapuka* **sp. nov.** (green squares, $n = 7$). Eigenvectors, eigenvalues, and percent of variation explained for the first two principal components are summarized in Table 6.

original description of *Liolaemus insolitus*, considered to be a widely distributed coastal species which reached altitudes above 2,000 m asl, including the populations of the “La Caldera batholith” from Department of Arequipa. Etheridge (1995), from the specimens considered by Boulenger (1885), identified the possible existence of a different species of *Liolaemus* from Department of Arequipa, which shows the characteristics of the specimens collected by Duellman (KU 163589, 3 km SW Uchumayo, at 2,150 m asl). During the following years, the regional museums of Peru considered the population from “La Caldera batholith” as an undescribed form associated with *Liolaemus insolitus* (Zeballos et al. 2002), which they called *Liolaemus* cf. *insolitus*. Nuñez (2004) identified the specimen considered by Boulenger (1885) as a new species of the genus *Phrynosaura* (synonym of *Liolaemus*). Gutiérrez and Quiroz (2010), based on photographic material, presumed that the population belonged to *L. cf. insolitus*. Later, Langstroth (2011) reviewed the field notes written by Duellman, Simmons, and Péfaur (unpublished) and their specimens cataloged as *Phrynosaura stolzmanni* from the University of Kansas (KU 163589, KU 163592, and KU 163594; collected from “10 km SE of the town of Uchumayo, in the La Caldera batholith”), and indicated that these lizards are not *Liolaemus stolzmanni*. Based on fieldnotes, which indicate that these specimens are individuals found in habitats of gray sand with granitic rocks and the coloration is cryptic with the habitat, he also highlights the mottled black, orange, and metallic blue back, and the lateral sides of the belly are orange; and these characters are corroborated with the photography of the individual KU163589; citing this population in his work as *Liolaemus* species 2 (KU 163589, KU 163592, and KU 163594). Finally, Abdala et al. (2020) corroborate through



Fig. 11. Phylogenetic trees showing the relationships between *Liolaemus anqapuka* sp. nov. and species within the *L. montanus* group by (A) Total Evidence analysis, (B) molecular phylogenetic analysis, and (C) morphological phylogenetic analysis. The values correspond to the support measure with symmetric resampling.

analysis of Total Evidence of the *L. montanus* group that the population from “La Caldera batholith” (*L. aff. insolitus*7) is an independent terminal, because it presents morphological characteristics different from the rest of the known species of *Liolaemus*. Therefore, we corroborate the hypothesis presented by Abdala et al. (2020), based in morphological and molecular phylogenetic evidence, which they named as *L. aff. insolitus*7.

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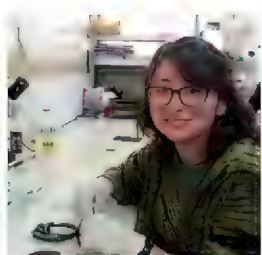
Literature Cited

- Abdala CS. 2007. Phylogeny of the *L. boulengeri* group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. *Zootaxa* 1538: 1–84.
- Abdala CS, Quinteros S. 2008. Una nueva especie de *Liolaemus* (Iguanidae: Liolemini) endémica de la sierra de Fiambalá, Catamarca, Argentina. *Cuadernos de Herpetología* 22: 35–47.
- Abdala CS, Quinteros AS, Espinoza RE. 2008. Two new species of *Liolaemus* (Iguania: Liolaemidae) from the Puna of northwestern Argentina. *Herpetologica* 64: 458–471.
- Abdala CS, Acosta JC, Cabrera MR, Villavicencio HJ, Marinero J. 2009. A new Andean *Liolaemus* of the *L. montanus* series (Squamata: Iguania: Liolaemidae) from Western Argentina. *South American Journal of Herpetology* 4: 91–102.
- Abdala CS, Juárez VI. 2013. Taxonomía y filogenia de un grupo de lagartos amenazados: el grupo de *Liolaemus anomalus* (Iguania: Liolaemidae). *Cuadernos de Herpetología* 27: 109–153.
- Abdala CS, Paz MM, Semhan RV. 2013. Nuevo *Liolaemus* (Iguania: Liolaemidae) con novedoso carácter morfológico, de la frontera entre Argentina y Chile. *Revista de Biología Tropical* 61(4): 1,563–1,584.
- Abdala CS, Quinteros AS. 2014. Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina: actualización taxonómica y sistemática de Liolaemidae. *Cuadernos de Herpetología* 28: 55–82.
- Abdala CS, Semhan RV, Laspiur A, Acosta JL. 2017. Rediscovery of *Liolaemus rabinoi* (Iguania: Liolaemidae) after 35 years: redescription, biological and phylogenetic information, and conservation challenges. *Salamandra* 53(1): 114–125.
- Abdala CS, Aguilar-Kirigin AJ, Semhan RV, Bulacios L, Valdes J, Paz MM, Gutiérrez R, Valladares P, Langstroth P, Aparicio J. 2019. Description and phylogeny of a new species of *Liolaemus* (Iguania: Liolaemidae) endemic to the south of the Plurinational State of Bolivia. *PLoS ONE* 14: e0225815.
- Abdala CS, Quinteros AS, Semhan RV, Bulacios AL, Schulte J, Paz MM, Ruiz-Monachesi MR, Laspiur A, Aguilar-Kirigin AJ, Gutiérrez R, et al. 2020. Unravelling interspecific relationships among highland lizards: first phylogenetic hypothesis using Total Evidence of the *Liolaemus montanus* group (Iguania: Liolaemidae). *Zoological Journal of the Linnean Society* 189(1): 349–377.
- Aguilar C, Wood PL Jr, Belk MC, Duff MH, Sites JW Jr. 2017. Different roads lead to Rome: integrative taxonomic approaches lead to the discovery of two new lizard lineages in the *Liolaemus montanus* group (Squamata: Liolaemidae). *Biological Journal of the Linnean Society* 120: 448–467.
- Aguilar-Puntriano C, Avila LJ, De la Riva I, Johnson L, Morando M, Troncoso-Palacios J, Wood PL Jr, Sites JW Jr. 2018. The shadow of the past: convergence of young and old South American desert lizards as measured by head shape traits. *Ecology and Evolution* 8: 11,399–11,409.
- Aguilar-Puntriano C, Ramírez C, Castillo E, Mendoza A, Vargas VJ, Sites JW. 2019. Three new lizard species of the *Liolaemus montanus* group from Perú. *Diversity* 11: 161.
- Aguilar-Kirigin A, Abdala CS. 2016. Primer registro de *Liolaemus puritamensis* Núñez and Fox, 1989 para

- el sur de Bolivia (Reptilia, Squamata, Liolaemidae). *Cuadernos de Herpetología* 30: 45–47.
- Aguilar-Kirigin AJ, Abdala CS, Aparicio J, Langstroth R. 2016. Primer registro de *Liolaemus pleopholis* Laurent, 1998 para Bolivia (Reptilia, Squamata, Liolaemidae). *Cuadernos de Herpetología* 30: 89–92.
- Avila LJ, Morando M, Sites JW Jr. 2006. Congeneric phylogeography: hypothesizing species limits and evolutionary processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata: Liolaemini). *Biological Journal of the Linnean Society* 89: 241–275.
- Boulenger GA. 1885. *Catalogue of the Lizards in the British Museum (Natural History). Volume II*. Second edition. Taylor and Francis, London, United Kingdom. 497 p.
- Breitman MF, Avila LJ, Sites JW Jr, Morando M. 2011. Lizards from the end of the world: phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini). *Molecular Phylogenetics and Evolution* 59: 364–376.
- Bruchmann I, Hobohm C. 2014. Factors that create and increase endemism. Pp. 51–68 In: *Endemism in Vascular Plants*. Editor, Hobohm C. Springer, Dordrecht, Germany. 348 p.
- Carrillo N, Icochea J. 1995. Lista taxonómica preliminar de los reptiles vivientes del Perú. *Publicaciones del Museo de Historia Natural Universidad Nacional Mayor de San Marcos* 49: 1–27.
- Cei JM, Péfaur JE. 1982. Una especie nueva de *Liolaemus* (Iguanidae: Squamata): su sistemática, ecología y distribución. Pp. 573–586 In: *Actas del VIII Congreso Latinoamericano de Zoología, Mérida, Venezuela, Octubre 1980*. Editor, Salinas PJ. Producciones Alfa, Mérida, Venezuela. 1,531 p.
- Chaparro JC, Quiroz AJ, Mamani L, Gutiérrez RC, Condori P, De la Riva I, Herrera-Juárez G, Cerdeña J, Arapa LP, Abdala CS. 2020. An endemic new species of Andean lizard of the genus *Liolaemus* from southern Perú (Iguania: Liolaemidae) and its phylogenetic position. *Amphibian & Reptile Conservation* 14(2) [General Section]: 47–63 (e238).
- Chávez RO, Moreira-Muñoz A, Galleguillos M, Olea M, Aguayo J, Latín A, Aguilera-Betti I, Muñoz AA, Manríquez H. 2019. GIMMS NDVI time series reveal the extent, duration, and intensity of “blooming desert” events in the hyper-arid Atacama Desert, northern Chile. *International Journal of Applied Earth Observation and Geoinformation* 76: 193–203.
- Corl A, Davis A, Kuchta S, Comendant T, Sinervo B. 2010. Alternative mating strategies and the evolution of sexual size dimorphism in the Side-blotched Lizard, *Uta stansburiana*: a population-level comparative analysis. *Evolution* 64: 79–96.
- De Queiroz K. 1998. The general concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. Pp. 57–75 In: *Endless Forms: Species and Speciation*. Editors, Howard D, Berlocher SH. Oxford University Press, New York, New York, USA. 552 p.
- De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
- Demangel D, Sepúlveda C, Jara M, Pincheira-Donoso D, Núñez H. 2015. *Liolaemus omorfi*, una nueva especie de lagarto de los andes del norte de Chile (Sauria, Liolaemidae). *Boletín del Museo Nacional de Historia Natural, Chile* 64: 139–155.
- Dixon J, Wright J. 1975. A review of the lizards of the iguanid genus *Tropidurus* in Perú. *Contribution in Science. The Natural History Museum of Los Angeles* 271: 1–39.
- Escomel E. 1929. Fauna de Arequipa. Obras Científicas. Lima. *Boletín de la Sociedad Geográfica de Lima* 37–38(1–4): 57–69.
- Etheridge R. 1995. Redescription of *Ctenoblepharis adspersa* Tschudi, 1845, and the taxonomy of Liolaeminae (Reptilia: Squamata: Tropiduridae). *American Museum Novitates* 3142: 1–34.
- Etheridge RE. 2000. A review of the *Liolaemus wiegmanni* group (Squamata, Iguania, Tropiduridae), and a history of morphological change in the sand-dwelling species. *Herpetological Monographs* 4: 293–352.
- Frost D. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania: Tropiduridae). *American Museum Novitates* 3033: 1–68.
- Goloboff P. 1993. Estimating character weights during tree search. *Cladistics* 9: 83–91.
- Goloboff P, Farris J, Nixon K. 2003. TNT: Tree analysis using new technology, v. 1.0. Available: <http://www.zmuc.dk/public/phylogeny/TNT/> [Accessed: 3 May 2020].
- Goloboff PA, Mattoni CI, Quinteros AS. 2006. Continuous characters analyzed as such. *Cladistics* 22: 589–601.
- Gutiérrez RC, Quiroz A. 2010. Herpetofauna del sur del Perú. Available: <http://herpetofaunadelsurdelperu.blogspot.com> [Accessed: 13 June 2020].
- Gutiérrez RC, Chaparro JC, Vásquez MY, Quiroz AJ, Aguilar-Kirigin A, Abdala CS. 2018. Descripción y relaciones filogenéticas de una nueva especie de *Liolaemus* (Iguania: Liolaemidae) y notas sobre el grupo de *L. montanus* de Perú. *Cuadernos de Herpetología* 32: 81–99.
- Gutiérrez R, Villegas L, López E, Quiroz A. 2010. Anfibios y reptiles de la Reserva Nacional de Salinas y Aguada Blanca, Perú. Pp. 219–226 In: *Diversidad Biológica de la Reserva Nacional de Salinas y Aguada Blanca*. Editors, Zeballos H, Ochoa J, López E. DESCO, INRENA, PROFONANPE, Lima, Peru. 313 p.
- Gutiérrez H, Castañeda R, Quipuscoa V, Peterson PM. 2019 *Aristida surperuanensis* (Poaceae, Aristidoideae), a new species from a desert valley in southern Perú. *Phytotaxa* 419(2): 182–188.

- Halloy M, Robles C, Salica MJ, Semhan R, Juárez V, Vicente N. 2013. Estudios de comportamiento y ecología de lagartijas de los géneros *Liolaemus* y *Phymaturus* (Iguania: Liolaemini). *Cuadernos de Herpetología* 27(1): 15–26.
- Irschick DJ, Losos JB. 1996. Morphology, ecology, and behavior of the Twig Anole, *Anolis angusticeps*. Pp. 291–301 In: *Contributions to West Indian Herpetology: a Tribute to Albert Schwartz*. Editors, Powell R, Henderson RW. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 457 p.
- IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. Available: <https://www.iucnredlist.org> [Accessed: 20 Jun 2020].
- IUCN. 2016. *A Global Standard for the Identification of Key Biodiversity Areas, Version 1.0*. 1st edition. IUCN, Gland, Switzerland. 46 p.
- IUCN. 2001. *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, United Kingdom. 30 p.
- Kier G, Barthlott W. 2001. Measuring and mapping endemism and species richness: a new methodological approach and its application on the flora of Africa. *Biodiversity and Conservation* 10: 1,513–1,529.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1,547–1,549.
- Langstroth R. 2011. On the species identities of a complex *Liolaemus* fauna from the Atiplano and Atacama Desert: insights on *Liolaemus stozmanni*, *L. reichei*, *L. jamesi pechecoi*, and *L. poconchilensis* (Squamata: Liolaemidae). *Zootaxa* 2809: 20–32.
- Laurent RF. 1983. Contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann (Iguanidae). *Boletín de la Asociación Herpetológica* 1: 16–18.
- Laurent RF. 1985. Segunda contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann Iguanidae. *Cuadernos de Herpetología* 1: 1–37.
- Lobo F, Espinoza RE. 1999. Two new cryptic species of *Liolaemus* (Iguania: Tropiduridae) from northwestern Argentina: resolution of the purported reproductive bimodality of *Liolaemus alticolor*. *Copeia* 1999(1): 122–140.
- Lobo F. 2005. Las relaciones filogenéticas dentro del grupo *chiliensis* (Iguania: Liolaemidae: *Liolaemus*): sumando nuevos caracteres y taxones. *Acta Zoológica Lilloana* 49: 65–87.
- Lobo F, Slodki D, Valdecantos S. 2010. Two new species of lizards of the *Liolaemus montanus* group (Iguania: Liolaemidae) from the northwestern uplands of Argentina. *Journal of Herpetology* 44(2): 279–293.
- Losos JB. 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards and evolutionary analysis. *Ecological Monographs* 60: 369–388.
- Lovett GM, Weathers KC, Sobczak WV. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. *Ecological Applications* 10: 73–84.
- Löwenberg-Neto P. 2014. Neotropical region: a shapefile of Morrone's (2014) biogeographical regionalization. *Zootaxa* 3802(2): 300.
- Málaga B, Díaz RD, Arias S, Medina CE. 2020. Una especie nueva de *Lasiurus* (Chiroptera: Vespertilionidae) del suroeste de Perú. *Revista Mexicana de Biodiversidad* 91: e913096.
- Manly BFL. 2000. *Multivariate Statistical Methods*. Chapman and Hall/CRC, Boca Raton, Florida, USA. 215 p.
- McCune B, Grace JB. 2002. *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, Oregon, USA. 300 p.
- McGarigal K, Cushman S, Stafford S. 2000. *Multivariate Statistics for Wildlife and Ecology Research*. 1st edition. Springer, New York, New York, USA. 283 p.
- Morrone JJ. 2014. Biogeographical regionalisation of the Neotropical region. *Zootaxa* 3782(1): 1–110.
- Noguera-Urbano EA. 2017. El endemismo: diferenciación del término, métodos y aplicaciones. *Acta Zoológica Mexicana* 33(1): 89–107.
- Núñez H. 2004. Cambios taxonómicos para la herpetofauna de Argentina, Bolivia y Chile. *Noticiario Mensual Nacional de Historia Natural de Chile* 353: 28–34.
- Núñez H, Yanez J. 1984. *Ctenoblepharis erroneus* sp. nov. de Iguanidae para la zona norte de Chile. *Museo Nacional de Historia Natural Boletín* 40: 91–95.
- Olave M, Avila LJ, Sites Jr JW, Morando M. 2014. Multilocus phylogeny of the widely distributed South American lizard clade *Eulaemus* (Liolaemini, *Liolaemus*). *Zoologica Scripta* 43: 323–337.
- Péfaur J, Dávila J, López E, Núñez A. 1978a. Distribución y clasificación de los anfibios del departamento de Arequipa. *Bulletin de l'Institut Français d'Études Andines* VII(1–2): 119–127.
- Péfaur JE, Davila J, Lopez E, Núñez A. 1978b. Distribución y clasificación de los reptiles del Departamento de Arequipa. *Bulletin de l'Institut Français d'Études Andines* VII(1–2): 129–139.
- Peres-Neto PR, Jackson DA. 2001. The importance of scaling of multivariate analysis in ecological studies. *Ecoscience* 8: 522–526.
- Quinn GP, Keough MJ. 2002. *Experimental Design and Data Analysis for Biologists*. 1st edition. Cambridge University Press, New York, New York, USA. 537 p.
- Quinteros AS, Abdala CS, Lobo FJ. 2008. Redescription of *Liolaemus dorbignyi* Koslowsky, 1898 and description of a new species of *Liolaemus* (Iguania: Liolaemidae). *Zootaxa* 1717: 51–67.
- Quinteros AS, Abdala CS. 2011. A new species of

- Liolaemus* of the *Liolaemus montanus* section (Iguania: Liolaemidae) from northwestern Argentina. *Zootaxa* 2789: 35–48.
- Quinteros AS. 2013. A morphology-based phylogeny of the *Liolaemus alticolor-bibronii* group (Iguania: Liolaemidae). *Zootaxa* 3670: 1–32.
- Quinteros AS, Ruiz Monachesi MR, Abdala C. 2020. Solving the *Liolaemus bibronii* puzzle, an integrative taxonomy approach: redescription of *L. bibronii* and description of three new species (Iguania: Liolaemidae). *Zoological Journal of the Linnean Society* 189(1): 315–348.
- Quipildor M, Abdala V, Santa Cruz Farfán R, Lobo F. 2018. Evolution of the cloacal and genital musculature, and the genitalia morphology in liolaemid lizards (Iguania: Liolaemidae) with remarks on their phylogenetic bearing. *Amphibia-Reptilia* 39: 101–111.
- Ramos VA. 2008. The basement of the Central Andes: the Arequipa and related terranes. *Annual Review of Earth and Planetary Sciences* 36: 289–324.
- Riveros-Riffo E, Torres-Mura JC. 2015. Distribución de *Liolaemus torresi* (Squamata: Liolaemidae), el lagarto endémico del desierto de Chile. *Gayana* 79(2): 220–222.
- Ruiz de Gamboa M, Ortiz-Zapata J. 2016. Los poros precloacales como diferenciadores de sexo en *Liolaemus* (Squamata: Liolaemidae): el caso de *L. jamesi* (Boulenger, 1891). *Gayana* 80(1): 125–128.
- Ruiz de Gamboa M, Correa C, Marambio-Alfaro Y, Riveros-Riffo E, Ortiz JC. 2018. Molecular evidence for conspecificity of two desert *Liolaemus* lizards (Iguania: Liolaemidae). *Zootaxa* 4438(2): 283–298.
- Ruiz S, Ruiz-Monachesi M, Abdala C. 2019. Revalidación de *Liolaemus choique* Abdala, Quinteros, Scrocchi y Stazzonelli, 2010 (Iguania: Liolaemidae). *Cuadernos de Herpetología* 33(1): 33–38.
- Schulte JA, Macey JR, Espinoza RE, Larson A. 2001. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biological Journal of the Linnean Society* 69: 75–102.
- Sokal RR, Rohlf FJ. 1998. *Biometry. The Principle and Practice of Statistics in Biological Research*. 3rd edition. Freeman, New York, New York, USA. 887 p.
- Troncoso-Palacios J, Ferri-Yáñez F. 2013. *Liolaemus patriciaiturrae* Navarro and Núñez, 1993 (Squamata: Liolaemidae): distribution extension in northern Chile and geographic distribution map. *Check List* 9: 78–80.
- Valdivia-Silva JE, Navarro-González R, Ortega-Gutiérrez F, Fletcher L, Perez-Montaña S, Condori-Apaza R, McKay C. 2011. Multidisciplinary approach of the hyperarid desert of Pampas de La Joya in southern Peru as a new Mars-like soil analogue. *Geochimica et Cosmochimica Acta* 75(7): 1,975–1,991.
- Valdivia-Silva JE, Navarro-González R, Fletcher L, Pérez-Montaña S, Condori-Apaza R, Ortega-Gutiérrez F, McKay C. 2012. Characteristics in the extreme hyper-arid region of Pampas de La Joya, Peru. Astrobiological approach in four years of observation: 2004–2008. *International Journal of Astrobiology* 11(1): 25–35.
- Valladares P, Etheridge R, Abdala CS. 2018. Resurrection and redescription of *Liolaemus reichei*, proposal of a neotype to stabilize its taxonomy. *Revista Mexicana de Biodiversidad* 89: 393–401.
- Van den Brink PJ, Van den Brink NW, Ter Braak CJF. 2003. Multivariate analysis of ecotoxicological data using ordination: demonstration of utility on the basis of various examples. *Australasian Journal of Ecotoxicology* 9: 141–156.
- Vargas HA, Hundsdoerfer AK. 2019. Two new native larval host plants of *Hyles annei* (Guérin-Méneville, 1839) (Lepidoptera, Sphingidae) in the Atacama Desert of northern Chile following exceptional summer rainfall. *Nota Lepidopterologica* 42(2): 151–156.
- Villamil J, Avila LJ, Morando M, Sites JW Jr, Leaché AD, Maneyro R, Camargo A. 2019. Coalescent-based species delimitation in the sand lizards of the *Liolaemus wiegmanni* complex (Squamata: Liolaemidae). *Molecular Phylogenetics and Evolution* 138: 89–101.
- Villegas-Paredes L, Huamaní-Valderrama L, Luque-Fernández C, Gutiérrez RC, Quiróz AJ, Abdala CS. 2020. Una nueva especie de *Liolaemus* (Iguania: Liolaemidae) perteneciente al grupo *L. montanus* en las lomas costeras del sur de Perú. *Revista de Biología Tropical* 68(1): 69–86.
- Zar JH. 2010. *Biostatistical Analysis*. 5th edition. Prentice Hall, Princeton, New Jersey, USA. 960 p.
- Zeballos P, López HE, Villegas L, Jiménez P, Gutiérrez R. 2002. Distribución de los reptiles de Arequipa, sur del Perú. *Dilloniana* 4: 27–34.



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Roberto C. Gutiérrez is a Biologist who graduated from the National University of San Agustín de Arequipa of Peru. Roberto is currently the Curator and Principal Researcher of the Herpetological Collection, Museum of Natural History, National University of San Agustín de Arequipa, Peru, and Vice President and Founding Member of the Herpetological Association of Peru (AHP). He is interested in the herpetofauna of the tropical Andes and the coastal desert, with a special focus on lizards of genus *Liolaemus*, and is developing studies in the systematics of amphibians and reptiles, ecology, and conservation. Roberto has conducted several biodiversity inventories, biological assessments, and biodiversity monitoring programs, and is currently working at the Natural Protected Areas Service of the Peruvian Ministry of Environment.



Álvaro Aguilar-Kirigin is a Bolivian Biologist who graduated from the Universidad Mayor de San Andrés, La Paz, has been a researcher at the Colección Boliviana de Fauna specializing in herpetology since 2002, and is a member of the Bolivian Network Researchers in Herpetology. He carried out two research internships in Argentina and Uruguay, focusing on the systematics and phylogeny of *Liolaemus* and the latitudinal patterns of seasonal changes in fat body size in 59 species of lizards. He has authored over 35 publications (18 of which were peer-reviewed), 10 book chapters, and seven technical cards as part of book chapters, including the descriptions of three species of *Liolaemus*. Álvaro is interested in integrative taxonomy, especially in the genus *Liolaemus*, because of its phenotypic plasticity in the Andean region. As a line of research, he is making progress with linear models in the study of classical comparative morphometry. Likewise, he is linked to the conservation of the wildlife that inhabit the Amazonian forest in the Department of Beni in Bolivia.



Wilson Huanca-Mamani is a Biologist from the Universidad de Concepción (Concepción, Chile), with a Doctorate in Plant Biotechnology from Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV), Unidad Irapuato, Mexico. Wilson is currently a researcher at the Universidad de Tarapacá (Arica, Chile). One of his research interests focuses on the population genetics of desert plants.



Pablo Valladares-Faúndez is a Biologist who graduated from the Austral University of Chile and obtained his Ph.D. from the University of Chile. Pablo is currently an academic in the Department of Biology, Science Faculty, University of Tarapacá, in northern Chile. He is interested in the study of vertebrates from arid and high Andean ecosystems, particularly lizards of the genera *Liolaemus* and *Microlophus*, and is developing studies on their taxonomy, systematics, ecology, and conservation. Pablo is also developing a herpetological collection of northern Chile.



José Cerdeña is a Biologist who graduated from the Universidad Nacional de San Agustín de Arequipa (Peru), and is a researcher at Museo de Historia Natural de la Universidad Nacional de San Agustín de Arequipa (MUSA) in Peru. José's research includes the systematics, taxonomy, and biogeography of Lepidoptera, but with a recent interest in the taxonomy and ecology of the genus *Liolaemus* in southern Peru.



Juan C. Chaparro is a Peruvian Biologist with extensive experience in studying the fauna of all the traditional geographic regions of Peru. Juan graduated in Biological Sciences from Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru; received a Master's degree in Biodiversity in Tropical Areas and Conservation in 2013, from an institutional consortium of the International University of Menéndez Pelayo (UIMP-Spain), Universidad Tecnológica Indoamérica (UTI-Ecuador), and Consejo Superior de Investigaciones Científicas (CSIC-Spain). He is currently the president of the Herpetological Association of Peru (AHP), director and curator of the Herpetological Collection of the Museo de Biodiversidad del Perú (MUBI, <https://mubi-peru.org/herpetologia/p-mubi>), and he also works as a consultant in environmental studies. Juan has authored or co-authored 51 peer-reviewed scientific papers, notes, book chapters, and books on various fauna (especially in herpetology and arachnology), on topics such as their taxonomy, biodiversity, systematics, phylogeny, conservation, and biogeography in South America. He is interested in those topics, as well as life history, distributional patterns, and evolution using amphibian and reptiles as biological models. Four species have been named in his honor: *Phyllomedusa chaparroi* (Amphibia), *Phrynosoma chaparroi* (Amphibia), *Hadruroides juanchaparroi* (Arachnida), and *Chlorota chaparroi* (Insecta).



Roy Santa Cruz is a Research Associate at Área de Herpetología del Museo de Historia Natural (MUSA), Universidad Nacional de San Agustín de Arequipa, Peru. His current research interests include the taxonomy, natural history, and conservation of amphibians and reptiles. He currently coordinates several research projects which focus on threatened species of Andean frogs.



Cristian S. Abdala is an Argentinian Biologist, a researcher at CONICET, and a professor at the Universidad Nacional de Tucumán (UNT) in Argentina. Cristian received his Ph.D. degree from UNT, and is a herpetologist with extensive experience in the taxonomy, phylogeny, and conservation of *Liolaemus* lizards. He has authored or co-authored over 70 peer-reviewed papers and books on herpetology, including the descriptions of 50 recognized lizard species, mainly in genus *Liolaemus*. One species, *Liolaemus abdalai*, has been named in his honor. He has conducted several expeditions through Patagonia, the high Andes, Puna, and the salt flats of Argentina, Chile, Bolivia, and Peru. Since 2016, Cristian has been the president of the Argentine Herpetological Association.

Appendix I. Specimens examined.

Liolaemus anqapuka **sp. nov.** ($n = 22$): **PERU. Arequipa:** Arequipa, Uchumayo: MUBI 13521–22, MUSA 4131, 4133–34; Arequipa, Uchumayo, Quebrada Tinajones, MUSA 1766–67, MUSA 4546, 5207–12, 5214, MUBI 14417, MUBI 14680, LSF 001, LSF 002; Arequipa, Uchumayo, between Quebrada Tinajones and Quebrada San Jose, MUSA 5573–75.

Liolaemus balagueri ($n = 18$): **PERU. Arequipa:** Camaná, Quilca, Lomas de Quilca, MUSA 1772–74, MUSA 5575–78, MUBI 13206–09, MUBI 16483–84, MUSM 39193–95; Camaná, Camaná, Lomas de La Chira, MUSM 39192, MUSA 5579.

Liolaemus chiribaya ($n = 11$): **PERU. Moquegua:** Mariscal Nieto, Torata, Jaguay Chico, MUSM 31548–50, MUSM 31553; Mariscal Nieto, Torata, Cerro los Calatos, MUSM 31547, MUSM 31386, MUSM 31388–91; Mariscal Nieto, between Moquegua and Torata, MUSM 31387.

Liolaemus etheridgei ($n = 17$): **PERU. Arequipa:** Cabrerías, Cayma, MUSA 501; Cerro Uyupampa, Sabandia, MUSA 549–54; Monte Ribereño de la Quebrada de Tilumpaya Chiguata. Pocsi, MUSA 1113–14, 1116, 1264–68, 1353; Anexo de Yura Viejo, Yura, MUSA 1229.

Liolaemus evaristoi ($n = 16$): **PERU. Huancavelica:** Los Libertadores, Pilpichaca, Huaytara, MUSA 2841 (holotype), 2781–85, 2840, 2842–45, MUBI 10474–78 (paratypes).

Liolaemus insolitus ($n = 10$): **PERU. Arequipa:** Lomas de Mejía, Deán Valdivia, MUSA 346, MUSA 1741, MUSA 2187–90; Alto Inclán, Mollendo MUSA 4787–88, MUSA 4812, MUSA 4815.

Liolaemus nazca ($n = 7$): **PERU. Ica:** Nazca, MUSM 31520–21, MUSM 31523, MUSM 31525–26, MUSM 31541, MUSM 16100.

Liolaemus poconchilensis ($n = 2$): **PERU. Tacna:** Morro Sama, Las Yaras, MUSA 1638–39.

Liolaemus polystictus ($n = 13$): **PERU. Huancavelica:** Mountain near Rumichaca, Pilpichaca, MUSA 1337–1338; Santa Inés, Castrovirreyna, MUSA 2448–2457; Santa Inés, FML 1683 (paratype).

Liolaemus robustus ($n = 11$): **PERU. Lima:** Surroundings of Huancaya, Reserva Paisajística Nor Yauyos Cochas, MUSA 1693–1702; Junín: Junín, FML 1682 (paratype).

Liolaemus signifer ($n = 12$): **PERU. Puno:** Titicaca Lake, 3,840 m, FML 1434; Titicaca Lake, road to Puno, FML 1557; near Tirapata, MUSA 1415; Huancané, Comunidad Taurahuta, MUSA 1441–43; Huerta Huayara community, 3 km before Puno, MUSA 1483–87.

Appendix II. Measured morphometric traits and meristic characters.

Morphological characters	<i>L. balagueri</i> $n = 12$	<i>L. chiribaya</i> $n = 10$	<i>L. insolitus</i> $n = 15$	<i>L. nazca</i> $n = 7$	<i>L. anqapuka</i> sp. nov. $n = 7$
SVL	51.08–64.96 58.82 ± 4.68	49.28–68.25 59.60 ± 6.59	47.35–65.77 56.79 ± 5.41	53.51–64.34 59.35 ± 4.98	52.15–73.53 60.14 ± 6.71
DN	1.03–2.04 1.31 ± 0.28	1.96–3.00 2.47 ± 0.30	0.91–1.96 1.53 ± 0.36	0.63–1.81 1.47 ± 0.42	0.96–1.68 1.36 ± 0.24
AH	3.59–5.61 4.45 ± 0.54	3.71–5.67 4.73 ± 0.66	3.21–5.06 4.23 ± 0.53	1.96–4.85 3.92 ± 0.93	4.16–5.43 4.70 ± 0.42
NC	1.65–2.91 2.09 ± 0.36	1.07–2.57 2.09 ± 0.52	1.52–2.85 2.09 ± 0.33	2.10–3.14 2.49 ± 0.38	2.10–2.73 2.47 ± 0.27
EO	6.11–8.96 7.49 ± 0.74	7.01–9.26 8.24 ± 0.72	7.12–8.88 7.90 ± 0.49	6.16–8.25 7.11 ± 0.80	7.00–9.62 8.54 ± 0.90
LEI	0.89–1.69 1.28 ± 0.26	0.88–1.28 1.09 ± 0.14	0.66–1.58 1.12 ± 0.26	0.47–2.06 1.31 ± 0.48	1.23–1.76 1.54 ± 0.21
PA	0.85–1.74 1.34 ± 0.26	1.31–1.72 1.43 ± 0.14	0.90–1.82 1.25 ± 0.26	0.51–1.91 1.24 ± 0.47	1.45–1.99 1.77 ± 0.21
AM	1.05–1.76 1.28 ± 0.20	2.00–2.86 2.46 ± 0.28	1.32–2.41 1.94 ± 0.47	0.46–1.31 1.06 ± 0.30	1.06–1.49 1.26 ± 0.18

A new species of *Liolaemus* from Peru

Appendix II (continued). Measured morphometric traits and meristic characters.

Morphological characters	<i>L. balagueri</i> <i>n</i> = 12	<i>L. chiribaya</i> <i>n</i> = 10	<i>L. insolitus</i> <i>n</i> = 15	<i>L. nazca</i> <i>n</i> = 7	<i>L. anqapuka</i> sp. nov. <i>n</i> = 7
LM	2.05–3.13 2.53 ± 0.34	0.84–1.55 1.20 ± 0.22	1.08–2.92 1.69 ± 0.66	1.23–2.64 2.16 ± 0.54	2.30–2.78 2.55 ± 0.21
NB	1.11–1.92 1.41 ± 0.23	1.19–1.63 1.42 ± 0.12	0.96–1.56 1.26 ± 0.18	1.16–1.87 1.56 ± 0.28	0.97–1.51 1.31 ± 0.17
HR	0.40–1.04 0.80 ± 0.17	0.64–1.22 0.86 ± 0.19	0.53–1.01 0.77 ± 0.11	0.69–1.54 0.93 ± 0.31	0.55–1.04 0.82 ± 0.15
ES	2.83–4.58 3.72 ± 0.49	3.20–4.06 3.57 ± 0.27	1.90–4.16 3.52 ± 0.53	2.93–6.62 3.93 ± 1.26	3.24–4.73 3.81 ± 0.49
hTy	1.69–2.63 2.16 ± 0.26	1.68–2.30 1.91 ± 0.21	1.02–2.09 1.72 ± 0.25	1.72–2.49 1.95 ± 0.26	1.37–2.02 1.78 ± 0.23
aTy	0.47–1.54 0.97 ± 0.26	1.18–1.65 1.37 ± 0.17	0.65–1.22 0.94 ± 0.20	0.67–1.13 0.94 ± 0.14	0.57–1.10 0.85 ± 0.18
LPO	0.91–1.67 1.20 ± 0.23	0.57–1.54 1.02 ± 0.32	0.53–1.49 1.17 ± 0.24	0.75–2.35 1.43 ± 0.50	0.60–1.07 0.89 ± 0.19
LPOT	0.43–0.85 0.61 ± 0.13	0.48–0.80 0.60 ± 0.11	0.37–0.72 0.52 ± 0.11	0.48–0.92 0.69 ± 0.15	0.33–0.82 0.55 ± 0.18
LCSP	1.01–2.00 1.52 ± 0.34	0.83–1.42 1.14 ± 0.19	0.54–1.52 1.03 ± 0.25	1.39–3.36 2.01 ± 0.67	0.66–1.37 1.03 ± 0.31
LCLB	0.68–1.56 1.15 ± 0.25	0.86–1.28 0.99 ± 0.12	0.55–1.31 0.97 ± 0.20	0.85–2.14 1.29 ± 0.46	0.57–1.30 1.03 ± 0.28
DEO	6.80–8.83 7.83 ± 0.67	7.31–9.32 8.26 ± 0.68	7.48–9.17 8.36 ± 0.55	6.90–8.67 7.58 ± 0.71	8.17–10.95 9.45 ± 1.03
1D	1.86–3.21 2.51 ± 0.39	1.84–3.12 2.52 ± 0.44	1.63–2.95 2.32 ± 0.31	1.61–2.82 2.13 ± 0.41	2.56–3.31 2.88 ± 0.30
G4D	1.10–1.59 1.30 ± 0.16	0.74–1.38 1.01 ± 0.21	1.17–2.04 1.53 ± 0.22	0.67–1.35 1.00 ± 0.23	1.29–2.11 1.54 ± 0.32
5D	2.89–3.84 3.29 ± 0.33	2.41–4.41 3.31 ± 0.56	2.44–3.40 2.84 ± 0.25	2.33–3.93 2.93 ± 0.52	2.28–3.41 3.00 ± 0.44
AHU	1.98–3.63 2.81 ± 0.51	1.99–4.58 3.03 ± 0.78	2.24–3.46 2.77 ± 0.38	2.01–3.93 3.06 ± 0.54	2.59–4.36 3.45 ± 0.60
LEA1	6.94–11.83 8.89 ± 1.40	8.65–10.81 9.75 ± 0.71	6.34–9.45 8.19 ± 0.86	7.01–8.95 8.17 ± 0.80	9.03–11.01 9.96 ± 0.68
AMU	3.76–5.28 4.54 ± 0.47	3.33–4.98 4.18 ± 0.60	2.67–4.68 3.71 ± 0.73	4.82–7.19 5.96 ± 0.79	3.60–5.80 4.43 ± 0.78
1P	2.87–3.68 3.19 ± 0.29	1.66–4.30 3.20 ± 0.86	2.50–3.78 3.15 ± 0.37	1.73–4.08 2.92 ± 0.72	2.51–3.42 3.08 ± 0.29
4U	0.93–2.06 1.45 ± 0.32	0.74–2.32 1.33 ± 0.45	0.98–1.77 1.30 ± 0.22	0.75–1.72 1.33 ± 0.36	0.97–1.87 1.45 ± 0.32
AL	16.19–20.03 17.43 ± 1.06	19.64–33.02 25.76 ± 4.97	12.12–19.74 15.99 ± 2.40	19.61–27.88 24.85 ± 2.70	15.27–24.37 19.85 ± 3.27
WTB	6.32–8.63 7.49 ± 0.76	6.19–9.15 7.76 ± 1.21	4.91–8.44 6.98 ± 1.07	6.24–9.20 7.46 ± 0.88	6.50–10.07 7.46 ± 1.20
ASPI	5.39–6.80 6.08 ± 0.44	4.37–7.80 6.45 ± 1.17	5.57–7.84 6.43 ± 0.69	2.70–7.20 4.55 ± 1.35	4.76–6.66 5.56 ± 0.64

Appendix II (continued). Measured morphometric traits and meristic characters.

Morphological characters	<i>L. balaguerei</i> <i>n</i> = 12	<i>L. chiribaya</i> <i>n</i> = 10	<i>L. insolitus</i> <i>n</i> = 15	<i>L. nazca</i> <i>n</i> = 7	<i>L. anqapuka</i> sp. nov. <i>n</i> = 7
LPI	4.01–6.12 5.07 ± 0.62	4.71–6.75 5.75 ± 0.76	3.73–6.40 5.03 ± 0.82	3.23–6.16 4.90 ± 0.87	5.20–9.22 6.33 ± 1.41
A11	4–8 6.33 ± 0.98	5–7 6.20 ± 0.63	5–9 6.27 ± 1.16	5–8 6.14 ± 1.07	6–8 7.00 ± 0.58
A12	6–8 7.08 ± 0.79	7–9 7.60 ± 0.70	7–8 7.47 ± 0.52	6–9 7.43 ± 0.98	7–10 8.43 ± 0.98
A15	6–8 6.67 ± 0.89	7–10 8.60 ± 0.97	7–9 7.80 ± 0.56	6–8 6.57 ± 0.98	8–10 9.00 ± 0.82
A13	5–7 6.08 ± 0.51	5–7 6.10 ± 0.57	5–8 6.40 ± 0.74	5–6 5.57 ± 0.53	6–8 6.86 ± 0.69
A19	5–7 5.67 ± 0.65	5–7 6.10 ± 0.57	5–8 6.27 ± 0.70	5–6 5.71 ± 0.49	7–8 7.14 ± 0.38
A14	4 4.00 ± 0.00	4–6 4.20 ± 0.63	4–6 4.67 ± 0.82	4–5 4.14 ± 0.38	4–5 4.14 ± 0.38
A16	6–8 6.67 ± 0.65	6–7 6.10 ± 0.32	6–8 7.07 ± 0.59	5–6 5.86 ± 0.38	6–7 6.14 ± 0.38
A17–1	7–9 7.50 ± 0.67	5–8 6.40 ± 1.07	7–8 7.20 ± 0.41	7–10 8.43 ± 0.98	8–10 9.00 ± 0.82
A18	12–16 13.75 ± 1.29	14–18 15.90 ± 1.20	14–18 15.07 ± 1.03	11–14 12.71 ± 1.11	13–17 14.29 ± 1.50
A20–1	7–8 7.33 ± 0.49	7–8 7.30 ± 0.48	6–9 7.67 ± 1.11	7–10 8.71 ± 1.11	7–9 8.29 ± 0.76
A20–2	9–11 10.17 ± 0.83	11–13 12.60 ± 0.84	8–16 12.07 ± 2.49	12–13 12.86 ± 0.38	9–13 11.29 ± 1.38
A20–3	14–16 14.67 ± 0.65	14–16 15.30 ± 0.67	12–16 14.40 ± 1.30	15–19 15.86 ± 1.57	11–15 13.57 ± 1.62
A20–4	12–18 15.33 ± 1.67	17–19 18.20 ± 0.92	10–17 12.73 ± 2.02	17–20 18.57 ± 1.13	15–18 17.00 ± 1.15
A20–5	8–11 9.58 ± 0.79	8 8.00 ± 0.00	6–10 7.73 ± 1.10	9–10 9.71 ± 0.49	7–10 8.71 ± 1.11
A21–1	5–10 8.17 ± 1.53	9–10 9.20 ± 0.42	6–11 7.80 ± 1.15	8–10 8.86 ± 0.90	7–11 9.29 ± 1.50
A21–2	10–13 11.83 ± 0.94	11–12 11.20 ± 0.42	10–12 10.93 ± 0.88	12–13 12.71 ± 0.49	11–14 12.00 ± 1.00
A21–3	9–18 15.00 ± 2.37	14–16 15.40 ± 0.70	12–16 14.00 ± 1.25	15–18 16.14 ± 1.21	12–18 15.14 ± 1.86
A21–4	19–24 20.33 ± 1.50	18–21 19.50 ± 0.85	20–22 20.67 ± 0.62	20–23 21.57 ± 0.98	20–23 21.43 ± 1.13
A21–5	10–14 11.58 ± 1.16	11–13 12.50 ± 0.71	10–12 11.27 ± 0.88	10–13 11.57 ± 1.51	9–13 10.86 ± 1.35
A22	52–56 53.50 ± 1.62	52–63 57.40 ± 3.50	58–69 63.40 ± 3.48	53–56 54.14 ± 1.35	60–76 67.29 ± 5.59
A26	0–7 3.00 ± 2.80	2–5 3.80 ± 1.03	0–8 4.20 ± 2.83	1–6 3.43 ± 1.51	2–6 3.43 ± 1.62

A new species of *Liolaemus* from Peru

Appendix II (continued). Measured morphometric traits and meristic characters.

Morphological characters	<i>L. balagueri</i> <i>n</i> = 12	<i>L. chiribaya</i> <i>n</i> = 10	<i>L. insolitus</i> <i>n</i> = 15	<i>L. nazca</i> <i>n</i> = 7	<i>L. anqapuka</i> sp. nov. <i>n</i> = 7
M2	1–2 1.33 ± 0.49	2 2.00 ± 0.00	1 1.00 ± 0.00	1–3 1.86 ± 0.69	1–2 1.86 ± 0.38
M3	6–9 7.50 ± 0.80	7–8 7.20 ± 0.42	5–9 7.07 ± 1.28	6–9 7.57 ± 1.13	7–8 7.43 ± 0.53
M5	3–5 4.25 ± 0.62	3–5 4.00 ± 0.47	4–8 6.73 ± 0.96	4–6 4.71 ± 0.76	5–6 5.29 ± 0.49
M4	3–6 4.75 ± 0.87	3–5 3.80 ± 0.63	3–8 6.47 ± 1.30	3–6 4.86 ± 1.07	4–7 5.71 ± 0.95
M13	1–6 3.92 ± 1.68	2–6 4.20 ± 1.40	5–16 10.00 ± 3.21	4–11 6.57 ± 2.64	3–8 5.29 ± 1.80
M14	2–6 3.75 ± 1.29	3–7 4.40 ± 1.07	2–8 4.27 ± 1.71	3–11 7.86 ± 2.97	1–6 3.86 ± 1.95
M15	1–6 3.50 ± 1.51	1–8 4.60 ± 2.67	5–24 12.53 ± 5.05	1–12 5.86 ± 3.72	1–4 2.29 ± 1.25
M23	26–30 27.17 ± 1.34	19–25 21.70 ± 1.89	26–32 28.80 ± 2.48	21–25 23.86 ± 1.46	28–36 30.86 ± 3.02
M26	52–60 56.50 ± 2.28	55–66 61.80 ± 3.68	52–60 55.80 ± 2.27	54–59 56.86 ± 1.95	63–72 67.29 ± 3.15
M32	65–79 73.17 ± 3.69	67–77 72.70 ± 2.95	69–80 73.53 ± 3.36	65–74 70.57 ± 2.88	73–87 82.43 ± 4.72
M34	1 1.00 ± 0.00	1 1.00 ± 0.00	2–4 2.87 ± 0.52	1–2 1.86 ± 0.38	1 1.00 ± 0.00
D6	6–8 6.92 ± 0.67	6–8 7.30 ± 0.67	6–8 6.47 ± 0.74	7–10 7.71 ± 1.11	7–9 8.14 ± 0.69

Note: Range in the first line; mean ± standard deviation (mm) for quantitative characters in the second line.

Legend: Snout-vent length (SVL); minimum distance between the nasal scales (DN); snout width at the edge of the canthal scale (AH); distance from the nose to the back edge of the canthal scale (NC); distance between the posterior edge of the superciliary series (EO); length of the interparietal (LEI); length of the parietal (PA); mental scale width (AM); length of the mental scale (LM); distance from nostril to mouth (NB); rostral height (HR); length of the subocular scale (ES); auditory meatus height (hTy); auditory meatus width (aTy); length of the preocular scale (LPO); preocular width (LPOT); length of the fourth supralabial scale (LCSP); length of the fourth lorilabial scale (LCLB); length between orbits (DEO); length of the first finger of the forelimb, without claw (1D); length of the claw of the fourth finger of the forelimb (G4D); length of the fifth finger of the forelimb without claw (5D); humerus width (AHU); distance from the insertion of the forelimb in the body toward the elbow (LEA1); thigh width (AMU); length of the first toe of the hind limb without claw (1P); length of the claw of the fourth toe of the hind limb (4U); length of the five dorsal scales in a row in the middle of the body (ED); cloacal opening width, measured distance between the corners of the cloaca (PP); body width (AL); width of the base of the tail (WTB); upper width of the pygal area (ASPI); length of the pygal area (LPI). Number of scales around the interparietal scale (A11); number of supralabials on the right side (A12); number of supralabials on the left side (A15); number of infralabials on the right side (A13); number of infralabials on the left side (A19); number of scales around the mental scale (A14); number of scales around the rostral scale (A16); number of lorilabials (A17–1); Hellmich index (A18); subdigital lamellae of the first finger of the forelimb (A20–1); subdigital lamellae of the second finger of the forelimb (A20–2); subdigital lamellae of the third finger of the forelimb (A20–3); subdigital lamellae of the fourth finger of the forelimb (A20–4); subdigital lamellae of the fifth finger of the forelimb (A20–5); subdigital lamellae of the first toe of the hind limb (A21–1); subdigital lamellae of the second toe of the hind limb (A21–2); subdigital lamellae of the third toe of the hind limb (A21–3); subdigital lamellae of the fourth toe of the hind limb (A21–4); subdigital lamellae of the fifth toe of the hind limb (A21–5); number of dorsal scales between the occiput and the level of the anterior edge of the thigh (A22); number of precloacal pores (A26); number of scales between canthal and nasal scales (M2); number of scales around the nasal scale (M3); number of supraocular enlarged scales in the right side (M5); number of supraocular enlarged scales in the left side (M4); number of organs in the postrostral scales (M13); number of organs in the third lorilabial scale (M14); number of organs in the scale above the row of the lorilabial scales and below the canthal and preocular scales (M15); number of gular scales (M23); number of scales around midbody (M26); number of ventral scales (M32); number of auricular scales, projecting scales on anterior edge of auditory meatus (M34); and number of paravertebral spots in the right side (D6).



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A new cryptic species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae), in the *C. littoralis* complex, from Anakkal, Palakkad, Kerala, India

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Abstract.—A new cryptic species of the gekkonid genus *Cnemaspis* is described from the Central Western Ghats of Kerala, India. *Cnemaspis palakkadensis* sp. nov. is a small-sized (snout-vent length less than 35 mm) *Cnemaspis* in the *littoralis* clade. Although the new species superficially resembles *C. littoralis*, it shows moderate levels of genetic divergence in the 16S rRNA gene, and can be differentiated from all other Indian congeners by a suite of distinct morphological characters: dorsal scales homogenous, small, smooth; absence of conical or spine-like tubercles on flanks; ventral scales smooth, imbricate; dorsal scales of limbs smooth; 15 or 16 femoral pores on each side separated by 14 poreless scales; lamellae under fourth digit of manus 12–15 and pes 14–17; absence of whorls of pointed tubercles on tail; median subcaudals enlarged, imbricate, smooth. The species is found in an ignored low-lying forest habitat in parts of the Anakkal reserve forest in Kerala.

Keywords. Asia, description, dwarf gecko, mountains, Reptilia, southern Western Ghats

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Introduction

The genus *Cnemaspis* Strauch, 1887 is among the most speciose of the Old World gekkotan genera, with at least 168 known species, ranking as the second most diverse gecko genus in the world after *Cyrtodactylus* (Uetz et al. 2020). Although large-scale molecular phylogenetic analyses have recently shown the genus to be polyphyletic (Gamble et al. 2012; Pyron et al. 2013; Zhang and Wiens 2016), there has been limited effort to resolve these issues, probably due to the lack of widespread sampling, specifically in the Indian region. With 48 species in mainland India, *Cnemaspis* represents the largest group of geckos in the country, with a large majority of the species restricted to the Western Ghats.

The Western Ghats, a long north-south orientated mountain chain extending from Gujarat in the north (21.00°N) to the southern tip of peninsular India in Tamil Nadu (08.25°N), is one of the 36 global biodiversity hotspots (Myers et al. 2000). Its historical isolation from

neighboring regions, complex topography, and humid tropical to subtropical climate have resulted in a high level of generic endemism, which is specifically accentuated in many amphibians and reptiles (Vijayakumar et al. 2014; Cyriac and Kodandaramaiah 2017). Widespread exploration in the higher reaches of the Western Ghats has rapidly increased the number of reptile and amphibian species in India (e.g., Zacharyah et al. 2011; Biju et al. 2014; Vijayakumar et al. 2014; Zacharyah et al. 2016; Sayyed et al. 2018; Chaitanya et al. 2019). However, recent surveys in the low-lying regions of the Western Ghats and several isolated hillocks in peninsular India are revealing a great amount of undocumented lizard diversity, especially among members of the genus *Cnemaspis* (Khandaker et al. 2019; Agarwal et al. 2020; Cyriac et al. 2020). In light of this, field surveys were conducted in the lowland forests bordering the Palghat gap in Kerala and Tamil Nadu, the largest geographical break in the long Western Ghats chain of mountains. These explorations revealed a new undescribed species

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which resembles the widespread *C. littoralis* (Jerdon) and is described here based on its genetic distinctiveness and a suite of distinct morphological characters.

Materials and Methods

Field sampling and specimens. Field surveys were conducted during May 2019, in parts of Anakkal, Palakkad District, Kerala, India. Specific sampling locations were chosen based on previous observations. All adult specimens were collected by hand, photographed in life, and then euthanized using halothane. Thigh muscles were collected as tissue samples for further genetic analysis, after which specimens were fixed in 4% formaldehyde for ~24 hours, washed in water, and transferred to 70% ethanol for long-term storage. Scalation and other morphological characters were recorded using a Lensel stereo microscope. The materials referred to in this study are deposited in the collection of the Bombay Natural History Society (BNHS), Mumbai, and were collected under the permits issued by the Kerala Forest and Wildlife Department (permits to RD, numbers WL10-41691/2014 and 94/2009).

Phylogenetic analysis. Total genomic DNA was extracted from the tissue samples using protocols as per Sayyed et al. (2016). The 16S rRNA mitochondrial gene was amplified using the primers designed by Palumbi et al. (1991) following standard 3-step PCR protocols (Palumbi 1996). The amplicons were then Sanger sequenced using the primers. The resulting sequences were manually checked for sequencing artifacts, and then added to the 16S rRNA sequence matrix generated by Cyriac et al. (2020) for the Indian *Cnemaspis*. However, *C. nilagirica* was removed from the matrix generated by Cyriac et al. (2020), since we found that the sample used was contaminated. The sequences were aligned using the MAFFT algorithm (Katoh and Standley 2013). The pair-wise uncorrected *p*-distances between and within species for the 16S rRNA gene were then calculated after removing all ambiguous positions for each sequence pair using MEGAX (Kumar et al. 2018). For the downstream phylogenetic analysis, multiple sequences of the same species were removed, except for the two sequences of *C. littoralis*. The best-fit substitution model was determined using PartitionFinder 2 (Lanfear et al. 2016) on the final 596 bp dataset and then a Maximum Likelihood analysis was performed using IQ-TREE (Nguyen et al. 2015) under the GTR+I+G substitution model with 1,000 standard bootstrap replicates. The MAFFT alignment, Partition Finder analysis, and Maximum Likelihood analysis were carried out using the phylogenetic workflow implemented in the PhyloSuite platform (Zhang et al. 2020). Following Cyriac et al. (2020), the tree was rooted by including three species of *Lygodactylus* and three species of *Phelsuma* as outgroups for the phylogenetic reconstruction (see Appendix 1).

Morphological and meristic data. For the specimens listed in Appendix 2, the following measurements were taken using a Yamayo digimatic calliper, a Mitutoyo 500, or a Tesacalip 64 (to the nearest 0.1 mm): snout-vent length (SVL), from tip of snout to anterior edge of cloacal opening; trunk length (TL), distance from axilla to groin measured from posterior edge of the forelimb insertion to the anterior edge of the hind limb insertion; trunk width (TW), maximum width of body; tail length (TAL), from vent to tip of tail; tail width (TLW), measured at widest point of tail; head length (HL), distance from tip of snout to posterior edge of mandible; head width (HW), maximum width of head; head depth (HD), maximum depth of head, from occiput to underside of jaws; upper arm length (UAL), distance from axilla to elbow; forearm length (FAL), from base of palm to elbow; femur length (FEL), distance from groin to the knee; tibia length (TBL), knee to tarsus; toe length (TOL), distance from tip of toe to the nearest fork; palm length (PAL), distance between posterior-most margin of palm and tip of fourth digit; finger length (FL), distance from the tip of the finger to the nearest fork; eye to nares distance (E-N), distance between anterior-most point of eye and nostril; eye to snout distance (E-S), distance between anterior-most point of eye and tip of snout; eye to ear distance (E-E), distance from anterior edge of ear opening to posterior corner of eye; tympanum diameter (EL), maximum distance end-to-end (height) of ear opening; distance between nares (IN), right to left nare; orbital diameter (OD), greatest diameter of orbit; interorbital snout distance (IO), distance between orbit and snout on frontal bone.

Meristic data recorded for all specimens were number of supralabials (SupL) and infralabials (InfL) on left (L) and right (R) sides; number of interorbital scales (InO); number of postmentals (PoM); number of posterior postmentals (PoP), scales that are surrounded by the posterior-postmentals and between infralabials; number of supranasals (SuN), excluding the smaller scales between the larger supranasals; number of the postnasals (PoN), all scales posterior to the naris; number of supraciliaries (SuS); number of scales between eye and tympanum (BeT), from posterior-most point of the orbit to anterior-most point of the tympanum; number of canthal scales (CaS), number of scales from posterior-most point of naris to anterior-most point of the orbit; number of dorsal paravertebral scales (PvS), between pelvic and pectoral limb insertion points along a straight line immediately left of the vertebral column; number of mid-dorsal scales (MbS), from the center of mid-dorsal row diagonally towards the ventral scales; number of midventral scales (MvS), from the first scale posterior to the mental to the last scale anterior to the vent; number of mid-body scales (BIS), across the ventral between the lowest rows of dorsal scales; femoral pores (FPores), the number of femoral pores; lamellae under digits of manus (MLam) and pes (PLam) on right (R) side, counted from

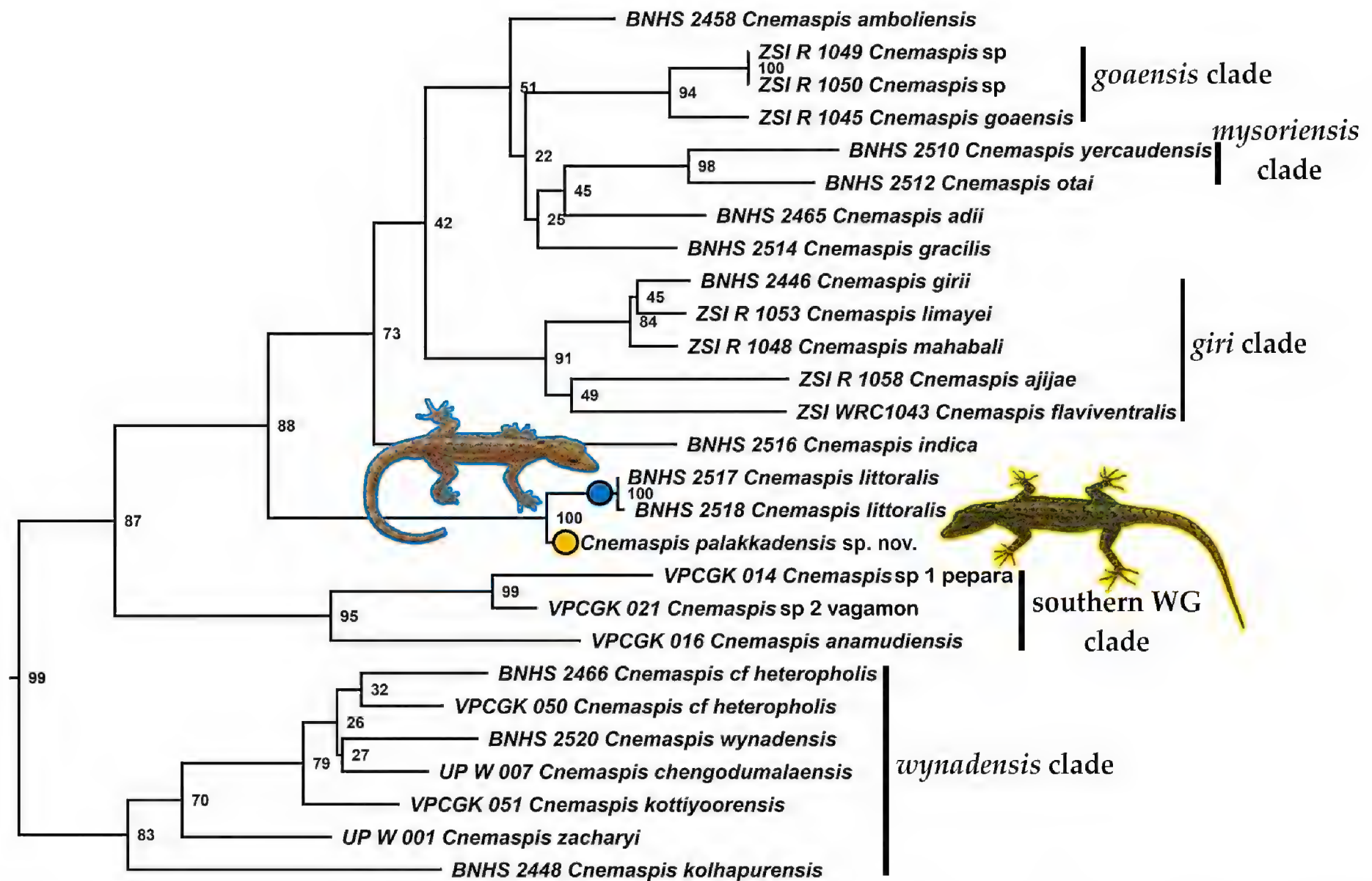


Fig. 1. 16S rRNA tree of the Indian *Cnemaspis* obtained from the Maximum Likelihood analysis in IQ-TREE. Node values indicate bootstrap support with values < 70 indicating low support, values between 70–90 indicating moderate support, and values > 90 indicating strong support. The blue and yellow circles represent the branches leading to *C. littoralis* and *C. palakkadensis* sp. nov., respectively, along with representative images of the two species indicated by their blue (*C. littoralis*) and yellow (*C. palakkadensis* sp. nov.) borders.

first proximal enlarged scansor greater than twice width of the largest palm scale, to distal-most lamella at tip of digits; and lamellae under fourth digit of pes (LampIV). For the geographical coordinates, altitude, and temperature readings, a Kestrel 4500 receiver was used. Opportunistic observations on the ecology of the species were also made during field work. Since the specimens from Palakkad most closely resembled *Cnemaspis littoralis*, specimens were compared with the neotype of *C. littoralis* and other associated material deposited at the Zoological Survey of India Western Ghats Regional Center (ZSI-WGRC), Kozhikode, India.

Morphometric analysis. The morphometric analysis was performed in R V. 3.5.2 (R Core Team 2016). A multivariate analysis was carried out on 25 morphometric variables. This analysis included only 25 variables for the analysis out of the 29 variables collected because some variables were unavailable for a few specimens due to missing tails or digits (see Table 3). A Principal Component Analysis (PCA) was performed on the 25 variables to identify the variables that contributed to the observed variation in the data. Plots were generated for the first and second, and the first and third principal components to visually examine the morphospace of

the new species and the morphologically similar *C. littoralis*.

Results

Phylogenetic Relationships

The topology recovered by the Maximum Likelihood analysis indicated well-supported deeper nodes but showed low support for many shallower nodes (Fig. 1). The topology was mostly consistent with the topology recovered by Sayyed et al. (2018) and Cyriac et al. (2010), except for the position of *Cnemaspis indica* where *C. indica* was sister to members of the *giri*, *gracilis*, *mysoriensis*, *goaensis*, and *amboliensis* clades. The new species was recovered as being sister to *C. littoralis* with very strong support (Fig. 1). The *littoralis* clade was sister to the (*indica* + (*giri* + (((*adii* + *mysoriensis*) + *gracilis*) + *goaensis*) + *amboliensis*))) clade. Uncorrected pairwise sequence divergence for the 16S rRNA gene indicated that the *littoralis* clade was deeply divergent from the rest of the species (sequence divergence > 10). However, there was only a moderate level of genetic divergence between *C. littoralis* and *C. palakkadensis* sp. nov., which ranged between 2.5–2.7%.

Table 1. Loadings obtained from the Principal Component Analysis of the 25 morphometric variables. Bold values indicate strong loading with correlation > 0.5.

Character	Description	PC1	PC2	PC3
SVL	Snout-vent length	-0.8278	-0.3045	-0.2238
TL	Axilla-groin distance	-0.8229	-0.2799	-0.2737
TW	Trunk width	-0.7241	-0.4259	-0.3070
OD	Eye diameter	0.6453	-0.6311	-0.0557
E-N	Eye-to-nasal distance	-0.9000	0.2800	0.0950
E-S	Snout length	-0.7350	-0.3478	-0.0852
E-E	Eye-to-ear distance	-0.3239	-0.5513	0.4186
IN	Inter-nasal distance	-0.9745	0.1924	-0.0163
EL	Horizontal diameter of ear opening	0.4675	-0.7923	-0.1568
HL	Head length	-0.6952	-0.5014	0.2935
HW	Head width	-0.8603	-0.3320	0.2034
HD	Head depth	-0.3677	-0.2852	0.5858
IO	Inter-orbital distance	-0.6162	-0.0902	-0.5711
UAL	Upper arm length	-0.4407	-0.7307	0.1714
FAL	Lower arm length	-0.6657	-0.4920	0.3509
PAL	Palm length	-0.7949	0.2111	-0.2336
FL1	Length of 1 st finger	0.5045	-0.5947	0.0675
FL3	Length of 3 rd finger	0.6421	-0.0028	0.3832
FL4	Length of 4 th finger	0.7575	-0.4670	0.0566
FL5	Length of 5 th finger	0.6749	-0.5265	-0.3075
FEL	Femur length	-0.9383	-0.2243	0.0515
TBL	Tibia length	-0.6979	-0.1892	0.2162
TOL1	Length of 1 st toe	0.7920	-0.5161	-0.0054
TOL2	Length of 2 nd toe	0.6670	-0.6226	-0.1914
TOL4	Length of 4 th toe	-0.4037	-0.5158	-0.4972
Eigenvalue		12.2328	5.0454	2.0250
Standard deviation		3.4976	2.2462	1.4230
Proportion of variance		0.4893	0.2018	0.0810
Cumulative proportion		0.4893	0.6911	0.7721

Morphometric Analysis

Principal Component Analysis indicated that the first three PCs explained 77.7% of the variation in morphology. PC1 explained ca. 48.9% of the variation and described a shorter, slender body form with a shorter head, shorter snout, larger eyes, and short limbs (Table 1). PC2 explained ca. 20.2% of the variation and is described mostly by smaller eyes, shorter eye-to-ear distance, smaller ear opening, and shorter forelimbs (Table 1). PC3 explained ca. 8.1% of the variation and described a less depressed head and narrower interorbital region (Table 1). Plots of PC1 with PC2 and PC1 with PC3 indicated considerable differences in the morphospace between the new species and *C. littoralis*, with the differences being along PC1 (Fig. 2A–B).

Systematics

Cnemaspis palakkadensis sp. nov.

Figs. 3–7; Tables 2–4.

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Holotype. BNHS 2790, an adult male, 32.2 mm SVL, from Anakal (10°52'50"N, 76°39'23"E; ca. 140 m asl), Palakkad District, Kerala, south-western India (Fig. 1), collected by Amit Sayyed, 18 May 2019.

Paratype. BNHS 2791, an adult male, 31.5 mm SVL, and BNHS 2792, an adult female, 34.1 mm SVL; collected from same locality as holotype by Vivek Vaidyanathan and Abhijit Nale, 19 May 2019.

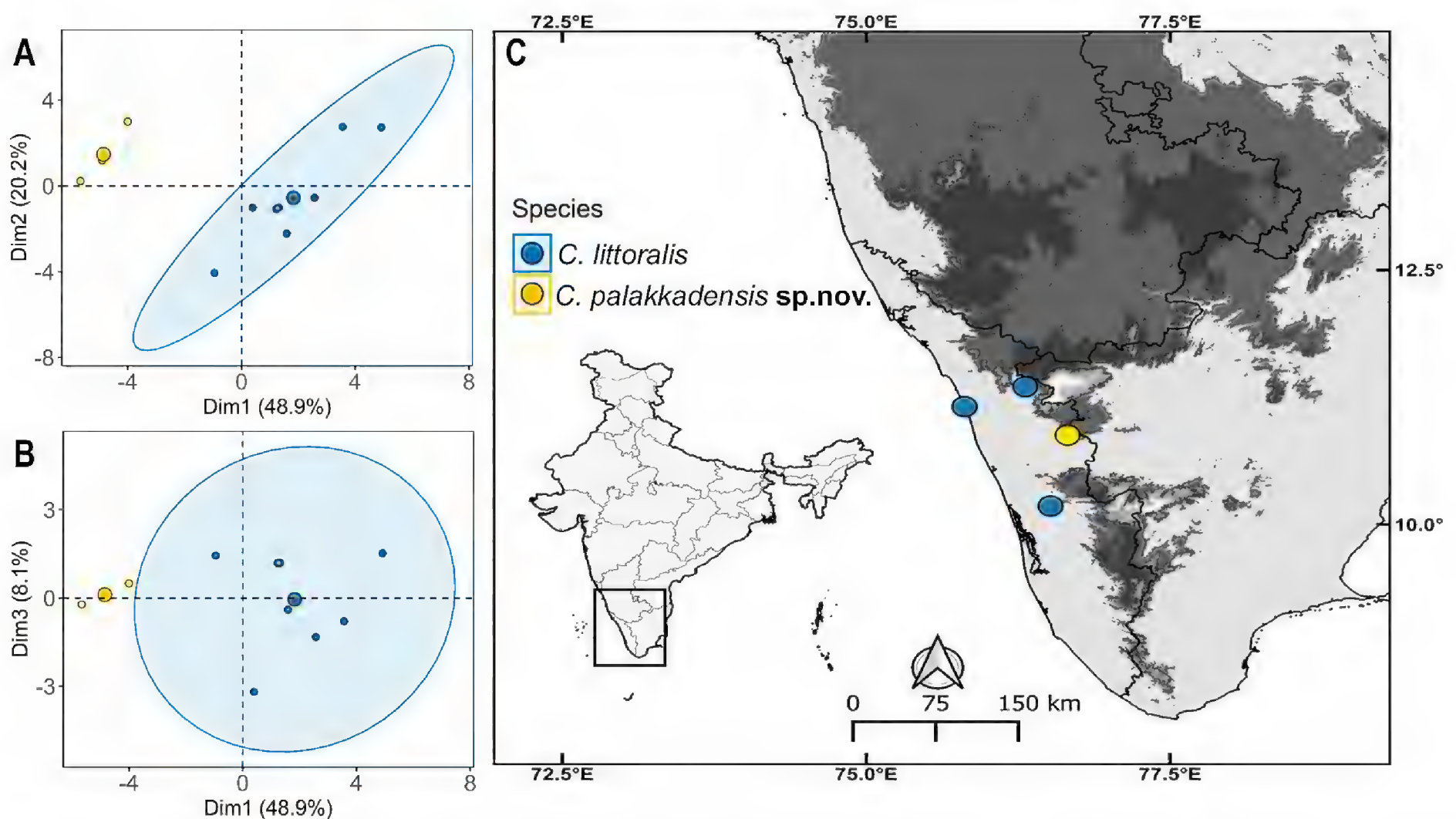


Fig. 2. Results of the morphometric analysis comparing the morphospace occupied by *Cnemaspis littoralis* and *C. palakkadensis* sp. nov. (A) Morphospace occupied by the two species as indicated by the first two dimensions (PC1 and PC2); (B) morphospace occupied by the two species as indicated by the first and third dimensions (PC1 and PC3); and (C) map showing the distributions of *C. littoralis* and *C. palakkadensis* sp. nov. The blue and yellow points in the morphospace and the map correspond to *C. littoralis* and *C. palakkadensis* sp. nov., respectively.

Diagnosis and comparison with Indian congeners. A small-sized *Cnemaspis*, SVL < 35 mm; dorsal pholidosis homogenous with small, smooth, granular scales in the vertebral and paravertebral regions; conical or spine-like tubercles absent on flank; ventral scales smooth, imbricate; males with 15–16 femoral pores on each thigh and no pre-cloacal pores; supralabials to angle of jaw 7–8, infralabials to angle of jaw 6–8; lamellae under fourth digit of manus 12–15, and pes 14–17; tail without whorls of enlarged tubercles; median subcaudals enlarged, imbricate, smooth, post cloacal spur absent in both sexes.

Cnemaspis palakkadensis sp. nov. can be distinguished from all other Indian congeners on the basis of the following differing or non-overlapping characters: Spine-like tubercles absent on flanks [versus spine-like tubercles present on flank in *C. amboliensis* Sayyed, Pyron, and Dileepkumar, *C. assamensis* Das and Sengupta, *C. anandani* Murthy, Nitesh, Sengupta, and Deepak, *C. flaviventralis* Sayyed, Pyron, and Dahanukar, *C. goaensis* Sharma, *C. gracilis* (Beddome), *C. jerdonii* (Theobald), *C. koynaensis* Khandekar, Thackeray, and Agarwal, *C. monticola* Manamendra-Arachchi, Batuwita, and Pethiyagoda, *C. mysoriensis* (Jerdon), *C. monticola* Manamendra-Arachchi, Batuwita, and Pethiyagoda, *C. nilagirica* Manamendra-Arachchi, Batuwita, and Pethiyagoda, and *C. otai* Das and Bauer]. Dorsal scales on midbody homogenous [versus heterogeneous in *C. aaronbaueri* Sayyed, Grismer, Campbell, and

Dileepkumar, *C. agarwali* Khandekar, *C. ajijae* Sayyed, Pyron, and Dileepkumar, *C. amba* Khandekar, Thackeray, and Agarwal, *C. amboliensis*, *C. anamudiensis* Cyriac, Johny, Umesh, and Palot, *C. anandani*, *C. andersonii* (Annandale), *C. australis* Manamendra-Arachchi, Batuwita, and Pethiyagoda, *C. avasabinae* Agarwal, Bauer, and Khandekar, *C. bangara* Agarwal, Thackeray, Pal, and Khandekar, *C. beddomei* (Theobald), *C. chengodumalaensis* Cyriac, Palot, and Deutiand Umesh, *C. flaviventralis*, *C. girii* Mirza, Pal, Bhosale, and Sanap, *C. goaensis*, *C. gracilis*, *C. graniticola* Agarwal, Thackeray, Pal, and Khandekar, *C. heteropholis* Bauer, *C. kottiyorensis* Cyriac and Umesh, *C. koynaensis*, *C. limayei* Sayyed, Pyron, and Dileepkumar, *C. maculicollis* Cyriac, Johny, Umesh, and Palot, *C. mahabali* Sayyed, Pyron, and Dileepkumar, *C. monticola* Manamendra-Arachchi, Batuwita, and Pethiyagoda, *C. nairi* Inger, Marx, and Koshy, 1984, *C. ornata* (Beddome), *C. shevaroyensis* Khandekar, Gaitonde and Agarwal, *C. sisparensis* (Theobald), *C. thackerayi* Khandekar, Gaitonde, and Agarwal, *C. wicksii* (Stoliczka), *C. yelagiriensis* Agarwal, Thackeray, Pal, and Khandekar, and *C. yercaudensis* Das and Bauer]. Presence of a series of 15–16 femoral pores on each side and the absence of pre-cloacal pores in males [versus absence of femoral pores in *C. aaronbaueri*, *C. anamudiensis*, *C. avasabinae*, *C. assamensis*, *C. beddomei*, *C. boiei* (Gray), *C. maculicollis*, *C. nairi*, and *C. ornata*; presence of both pre-cloacal and femoral pores in *C. adii*, *C.*

Table 2. Measurements (to the nearest 0.1 mm) of the type series of *Cnemaspis palakkadensis* **sp. nov.** Measurement abbreviations are defined in the text.

Measurement	Holotype	Paratypes	
	BNHS 2790	BNHS 2791	BNHS 2792
Sex	male	male	female
SVL	32.2	31.5	34.0
TL	15.6	14.6	16.7
TW	6.4	5.2	6.8
TAL	35.7	32.6	33.3
TLW	3.4	2.7	2.8
HL	8.9	8.7	9.1
HW	5.2	5.1	5.2
HD	3.3	3.3	3.5
UAL	4.5	3.8	4.6
FAL	5.5	5.3	5.6
FEL	5.8	5.7	5.9
TBL	5.9	5.9	6.0
PAL	3.7	3.6	3.9
E–N	4.0	4.0	4.2
E–S	4.1	4.1	4.3
E–E	2.5	2.5	2.5
EL	0.3	0.2	0.3
IN	1.6	1.6	1.6
OD	1.2	1.2	1.2
IO	3.3	3.2	3.4

agarwali, *C. amboliensis*, *C. andersonii*, *C. australis*, *C. bangara*, *C. gracilis*, *C. goaensis*, *C. graniticola*, *C. mysoriensis*, *C. otai*, *C. shevaroyensis*, *C. thackerayi*, *C. wicksii*, *C. yelagiriensis*, and *C. yercaudensis*]; and from the following species by presence large number of femoral pores [versus < 10 femoral pores on each side in *C. aijiae*, *C. amba*, *C. anandani*, *C. chengodumalaensis*, *C. flaviventralis*, *C. girii*, *C. heteropholis*, *C. indica*, *C. jerdonii*, *C. kottiyorensis*, *C. koynaensis*, *C. limayei*, *C. mahabali*, *C. nilagirica*, *C. sisparensis*, *C. wynadensis*, and *C. zacharyi* Cyriac, Palot, and Deutiand Umesh; and a continuous series of precloacal-femoral pores in *C. kolhapurensis*]. Median subcaudals enlarged [versus small median subcaudals in *C. adii*, *C. aijiae*, *C. amba*, *C. andersonii*, *C. flaviventralis*, *C. girii*, *C. gracilis*, *C. koynaensis*, and *C. limayei*].

Cnemaspis palakkadensis **sp. nov.** could be confused with the morphologically similar *C. littoralis* (Jerdon), but can be distinguished by its longer trunk length (AG 46–49% of SVL versus AG 37–46% of SVL in *C. littoralis*); much smaller eyes (ED 13–14% of HL versus ED 16–21% of SVL in *C. littoralis*); absence of conical or spine-like tubercles on flanks (versus small spine-like tubercles present on flanks in *C. littoralis*); supralabials to angle of jaw 7–8 (versus 9–10 supralabials in *C. littoralis*); number of scales between eye and tympanum 18–19 (versus 17); mid-dorsal scales 54–57 (versus 52);

midventral scales 130–134 (versus 122); number of mid-body scales 32–38 (versus 26); absence of a small post-cloacal spur on both sides of the tail and absence of whorls of enlarged tubercles on the tail (versus a single post-cloacal spur present on each side of the tail and whorls of small but enlarged tubercles on the dorsal side of the tail in *C. littoralis*).

Description of holotype. An adult male of SVL 32.2 mm (Fig. 3A–B); head moderately short (HL 17.6% of SVL), narrow (HW 15.9% of SVL), flat (HD 59.4% of HL), distinct from neck; snout short (E–S 78.6% of HL), slightly curved laterally; scales on snout granular, smooth, larger than those on the forehead and interorbital region (Fig. 4A); eye small (OD 21.8% of HL); pupil rounded; 13 supraciliaries; 30 interorbital scales; ear opening small (EL 5.7% of HL), longer than broad; 18 scales between eye and tympanum. Rostral wider than long, partially divided by a deep median groove; nostrils small, bordered posteriorly by two small, granular, postnasal scales; single enlarged supranasal on each side separated by an elongated intermediate scale. Mental large, triangular, not pointed posteriorly, broader than long, bordered posteriorly by two postmentals and a single intermediate chin shield broadly separated the postmentals, eight scales surrounded posteriorly by the posterior postmentals, infralabials, and the mental;

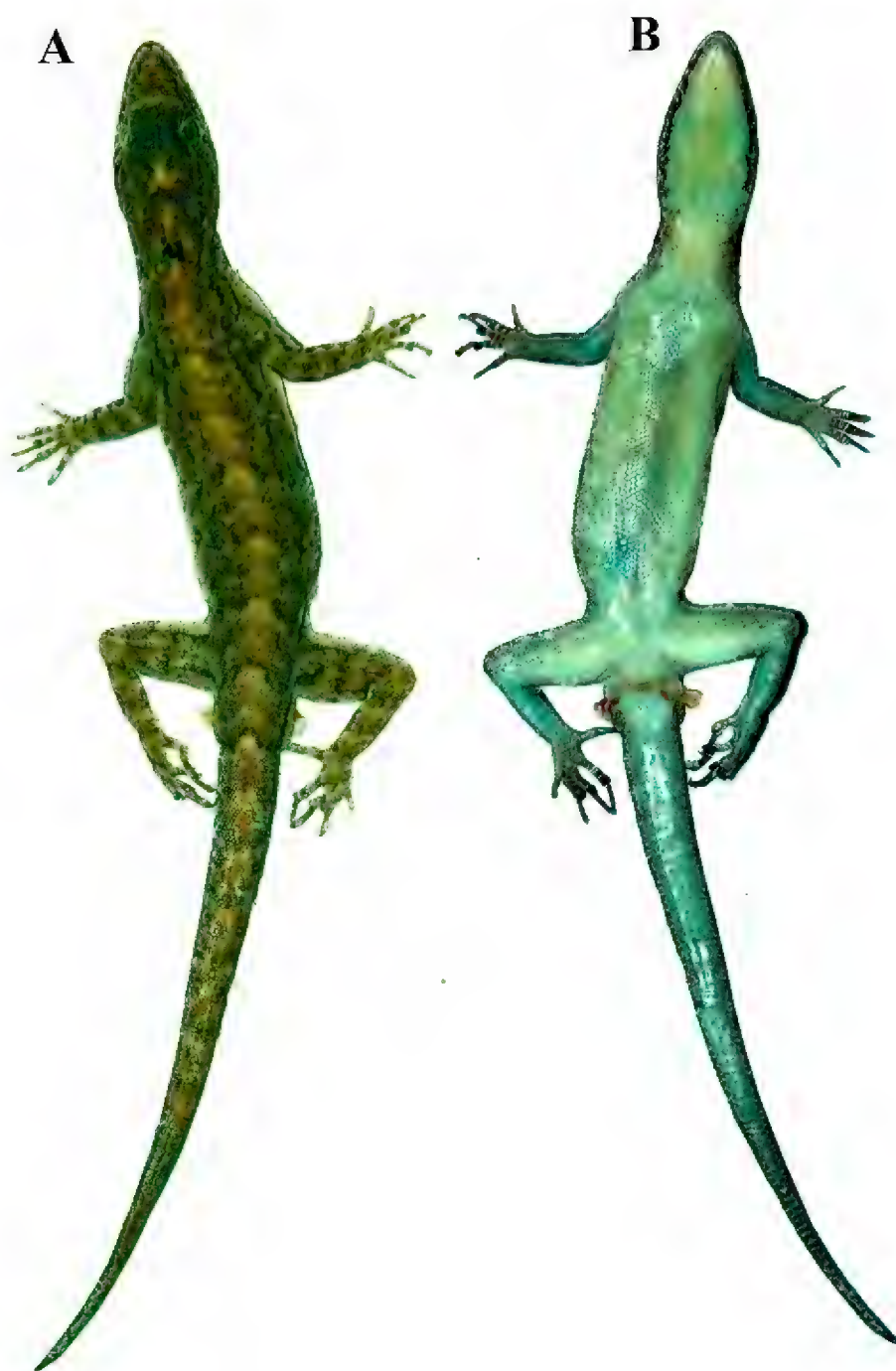


Fig. 3. Holotype (BNHS 2790) of *Cnemaspis palakkadensis* sp. nov. (A) Dorsal view and (B) ventral view of full-body. Photos by Amit Sayyed.

three smooth, large scales posteriorly surrounded by intermediate chin shield; gular scales granular, smooth, larger than those on throat (Fig. 4B). Seven supralabials to angle of jaw on each side, supralabial I largest, decreasing in size posteriorly; six infralabials to angle of jaw on each side, infralabial I largest, decreasing in size posteriorly (Fig. 4C).

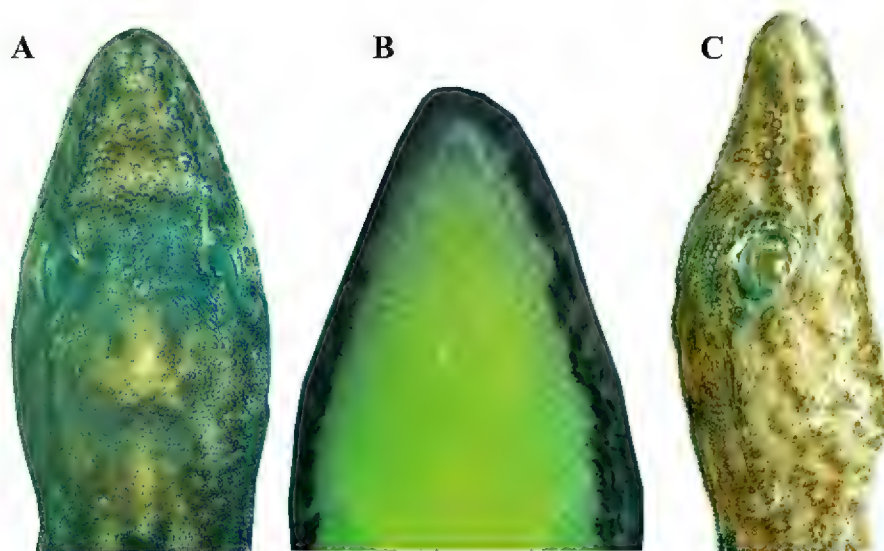


Fig. 4. Holotype (BNHS 2790) of *Cnemaspis palakkadensis* sp. nov. (A) Dorsal, (B) ventral, and (C) lateral views of the head. Photos by Amit Sayyed.

Body slender, short (TL 40%) without conical or spine-like tubercles on flanks (Fig. 5A, C). Dorsal scales of the body and flank homogenous, small, granular, smooth; scales on forehead, neck, and dorsal body equal in size; paravertebral scales 112; number of mid-dorsal scales 54; scales arranged in 33–35 longitudinal rows at midbody, number of midventral scales 131, smooth, imbricate, larger than dorsals (Fig. 5B). Fore and hind limbs relatively long, slender (FL 16.8%; TBL 16.4%); dorsal scales of brachium granular, smooth, larger than forearm; dorsal scales of forearm small, granular; ventral scales of brachium and forearm small, smooth; dorsal scales on palm, foot and fingers granular, smooth; scales on palmar and plantar surfaces smooth; subdigital lamellae entire, few fragmented; series of unpaired lamellae on basal portion of digits, separated from fragmented distal lamellae by a single large scale at the inflection; proximal lamellae series: 1-3-4-4-3 (right manus), 1-4-5-6-3 (right pes); distal lamellae series: 9-11-14-14-12 (right manus), 9-13-15-17-11 (right pes). Relative lengths of digits, fingers: IV (2.71 mm) > III (2.53 mm) > II (2.42 mm) > V (1.87 mm) > I (1.18 mm); toes: IV (3.68 mm) > III (3.16 mm) > II (1.99 mm) > V (1.75 mm) > I (0.85 mm) [Fig. 6A–B]. Femoral pores 15; 14 poreless scales between right and left femoral pore series; three rows of enlarged, roughly hexagonal scales above the femoral pores, larger than those on precloacal region; no precloacal pores; precloacal scales equal in size to the belly scales (Fig. 6C).

Tail long (TAL 111%), cylindrical, base swollen; post-cloacal spur absent on each side of lateral surface of hemipenial bulges at base of tail; dorsal scales of tail homogenous, smooth, granular, without enlarged, conical tubercles forming whorls, ventral scales imbricate, smooth; median subcaudals enlarged, smooth; those at the base are moderately smaller and imbricate (Fig. 6D–F).

Coloration in life (Fig. 6A–C). Male and female of the new species are the same in dorsal appearance. Dorsum of head mottled with brown and yellow; ventral side of head bright orange-yellow in males but white in females, bordered by a dark brown line up to the throat; nape with a small, black ocelli-like marking. Iris yellow with thin dark yellow line bordering pupil; pupil circular, black; supraciliaries yellow; supralabials and infralabials yellow. Dorsum of the body and limbs dull grey with brown and pale yellowish mottling; vertebral region pale yellow with 6–7 dark-edged lighter markings. Tail dull brown dorsally, with irregular faded yellow spots. Ventral side of body and tail white.

Coloration in preservative. Dorsum of the body and limbs with brown which turns into dark brown and pale yellow mottling and into grey; ventral side of body and tail greyish white; ventral side of head in males grey with slight yellowish tinge.

Table 3. Meristic data for the type series of *Cnemaspis palakkadensis* **sp. nov.** The symbol “?” indicates a broken finger, and “–” indicates pores not present.

Character	Holotype (BNHS 2790)	Paratype (BNHS 2791)	Paratype (BNHS 2792)
Sex	male	male	female
SupL R/L	7/7	8/8	8/8
InfL R/L	6/6	8/8	8/7
SuS	13	13	13
InO	30	32	31
BeT	18	18	19
PoN	2	2	2
PoM	2	2	2
PoP	8	10	8
SuN	1	1	1
CaS	14	15	14
PvS	112	109	113
MbS	54	54	57
MvS	131	130	134
BIS	33–35	32–34	35–38
FPores	15/15	16/16	–
MLam R	9-11-14-14-12	7-10-12-12-11	9-?-13-15-13
PLam R	9-13-15-17-11	7-12-?-14-13	9-14-16-17-15

Variation of the type series (Tables 1–3). The SVL of adult specimens in the type series of *Cnemaspis palakkadensis* **sp. nov.** ($n = 3$) ranges from 31.5 to 34.1 mm; number of posterior postmentals, 8–10; scales between eye and tympanum, 18–19; number of interorbitals, 30–32; number of canthal scales, 14–15; number of dorsal paravertebral scales, 109–113; number of mid-dorsal scales, 54–57; number of midventral scales from mental to cloaca, 130–134; number of mid-body scales across belly, 32–38; lamellae under fourth digit of manus (MLamIV) and pes (PLamIV), 12–15 and 14–17, respectively. Male and female paratypes match the holotype in overall coloration, except for the coloration on the throat.

Etymology. The specific epithet *palakkadensis* refers to the Palakkad district, from which the type series was collected.

Suggested Common Name. Palakkad Dwarf Gecko.

Distribution. At present, the new species is only known from the type locality in Anakal reserve forest (10°52'50"N 76°39'23"E) in Palakkad District of Kerala state (Fig. 1C), which is a low-land moist deciduous to riparian forest at an elevation of 84–170 m asl on the northern border of the Palghat gap, a ca. 30-km gap separating the central and southern Western Ghats. However, it is possible that the range of this species may extend to other low-land forests in the Palakkad region of Kerala and Coimbatore of Tamil Nadu.

Natural history. The species is found in low-land moist deciduous to semi-evergreen forest habitat of Palakkad hills of the Central Western Ghats. The climate of the region is moist and humid, and the area is rich in natural forest. All the specimens were found active during the day on the trunks, branches, and exposed roots of large trees around small streams (Fig. 8A–B), suggesting that this species is arboreal and diurnal. Single eggs or pairs of eggs were observed in several tree holes during the field survey (Fig. 7D). Two eggs that were collected measured 5.1×4.9 mm and 5.2×5.0 mm. The types were found sympatrically with *Ophiophagus hannah* (Cantor), *Trimeresurus gramineus* (Shaw), *Naja naja* (Linnaeus), *Hypnale hypnale* (Merrem), *Ahaetulla nasuta* Lacepede, *Amphiesma stolatum* (Linnaeus), *Lycodon aulicus* (Linnaeus), *Dendrelaphis tristis* (Daudin), *Cnemaspis gracilis*, *Cnemaspis* sp., *Psammophilus dorsalis* (Gray), and *Psammophilus* sp. (Stoliczka).

Discussion

The phylogenetic analysis recovered a topology mostly concordant with previous studies employing the 16S rRNA gene (Sayyed et al. 2018; Cyriac et al. 2020), even after the removal of *Cnemaspis nilagirica* from the sequence matrix. However, there was a difference in the phylogenetic placement of *Cnemaspis indica*. While this analysis recovered moderate support for a sister relationship between *C. indica* and members of the *giri*, *gracilis*, *mysoriensis*, *goaensis*, and *amboliensis* clades (Fig. 1), Sayyed et al. (2018) and Cyriac et al. (2020)

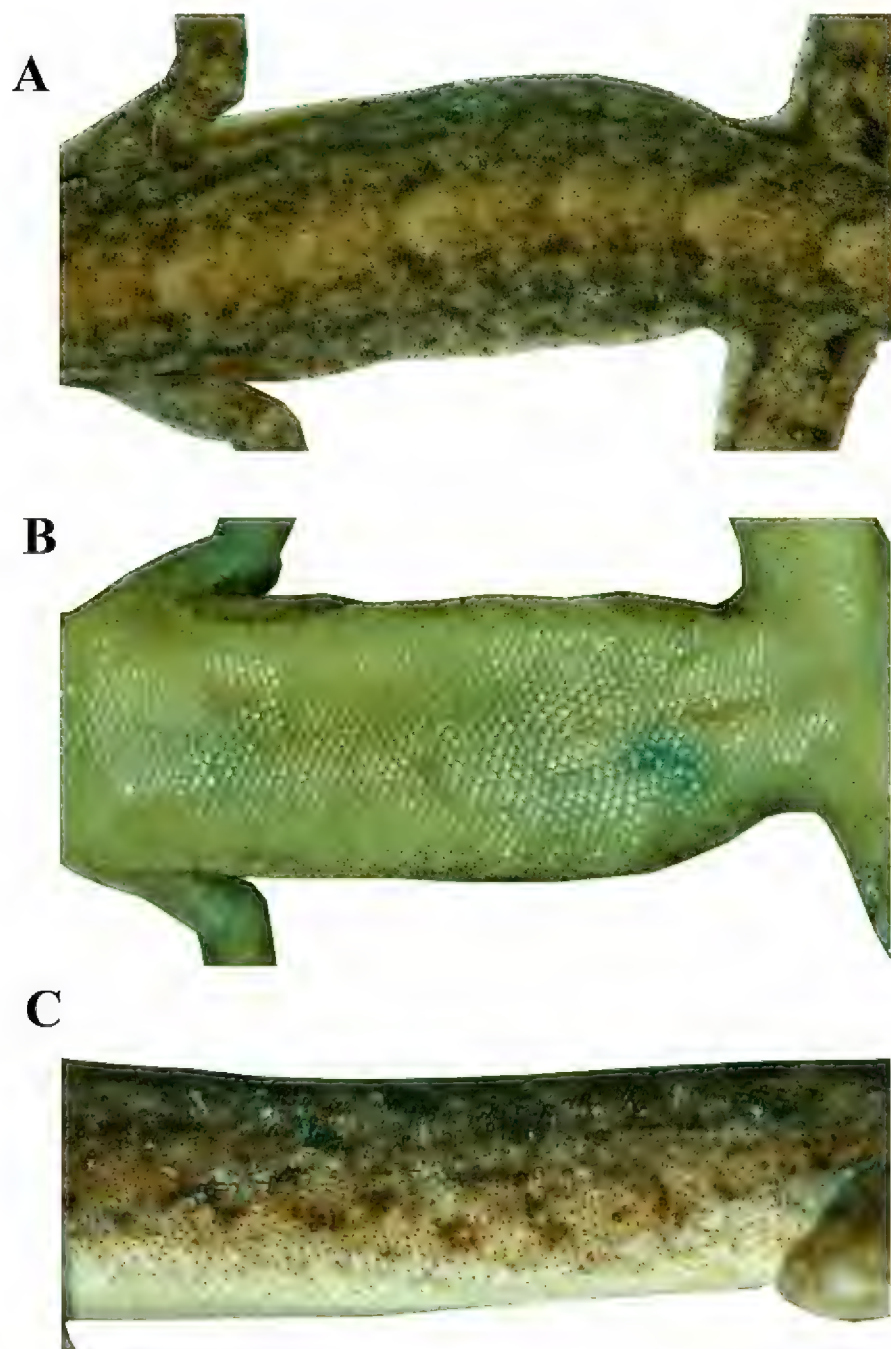


Fig. 5. Holotype (BNHS 2790) of *Cnemaspis palakkadensis* **sp. nov.** (A) Dorsal pholidosis at midbody, (B) ventral scales at midbody, (C) scales on lateral surface of trunk. Photos by Amit Sayyed.

recovered a sister relationship between *C. indica* and members of the *gracilis*, *mysoriensis*, *goaensis*, and *amboliensis* clades, albeit with very low support. Such discordance could be due to the removal of *C. nilagirica* from the analysis or the differences in our analytical approach. Nonetheless, our analysis indicates slightly improved support values for deeper nodes compared to earlier 16S rRNA trees.

The analysis clearly indicated that *Cnemaspis palakkadensis* **sp. nov.** was sister to *C. littoralis*, and showed moderate levels genetic divergence (2.5–2.7%) from the latter. Although genetic divergences of 2–4% are considered low for the 16S rRNA gene, morphologically distinct species have been shown to exhibit shallow genetic divergence (ca. 1%) for the 16S rRNA gene (Shanker et al. 2017). The specimens described here as *C. palakkadensis* **sp. nov.** occupy a distinct morphospace compared to *C. littoralis*, despite the superficial morphological resemblance and shallow genetic divergence with *C. littoralis*. Further, the genetic divergence between *C. palakkadensis* **sp. nov.** and *C. littoralis* was greater than the average intraspecific genetic divergence estimated for 16 *Cnemaspis* species based on the 16S rRNA gene of only $0.4 \pm 0.42\%$, with the maximum intraspecific genetic divergence recorded

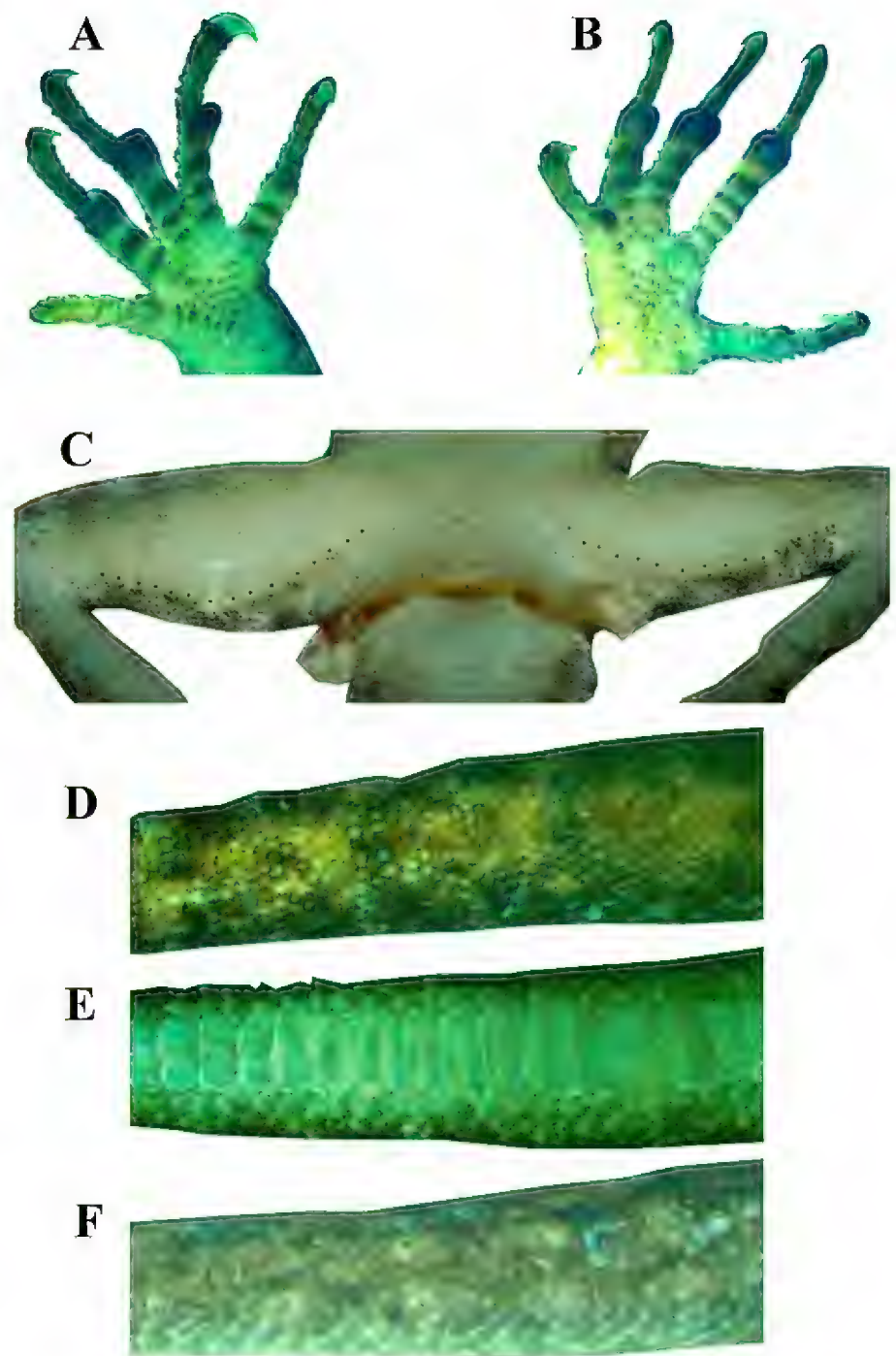


Fig. 6. Holotype (BNHS 2790) of *Cnemaspis palakkadensis* **sp. nov.** (A) Lamellae on manus, (B) lamellae on pes, (C) femoral pores, (D) dorsal scalation of tail, (E) subcaudals, (F) lateral side of tail. Photos by Amit Sayyed.

being 1.7% (see Appendix 3). The two species are also morphologically distinct and can be distinguished based on several non-overlapping diagnostic characters.

Interestingly, *C. palakkadensis* **sp. nov.** was found in low-land moist deciduous to semi-evergreen forests in the northern border of the Palghat gap. This gap forms a major dispersal barrier and biogeographic divide to many groups of animals which are distributed on the higher reaches of the Western Ghats (Robin et al. 2010; Van Bocxlaer et al. 2012; Vijayakumar et al. 2014). However, our understanding of what biogeographic barriers influence the distribution of low-land habitat-specialist species remains poor, mostly due to the lack of systematic exploration of low-lying regions. The discovery of *C. palakkadensis* **sp. nov.** from the low-land forests in the Palghat gap further highlight the presence of unknown diversity within the species *C. littoralis*, which is thought to have a wide distribution in the littoral regions of Kerala (Cyriac and Umesh 2013). However, widespread systematic explorations in these low-land forests will be necessary to determine the distributional range of this species. Cyriac and Umesh (2013) designated a neotype for *C. littoralis* based on specimens



Fig. 7. Color in life of *Cnemaspis palakkadensis* **sp. nov.** (A) Holotype male (BNHS 2790), (B) paratype female (BNHS 2792), (C) holotype male (BNHS 2790) showing the coloration of throat, (D) egg. Photos by Amit Sayyed.

collected from Chaliyam coast in Kozhikode, Kerala, and reported additional specimens from Narayamkulam (= Chengodumala) in Kozhikode district, Nellikuth in Malapuram district, and Kapprikkad in Ernakulam district of Kerala. They also reported observations of *C. littoralis* from Kannur, Thrissur, Palakkad, Ernakulam, and Thiruvananthapuram in Kerala. However, given the possibility of cryptic species within this group, the true distribution of *C. littoralis* will need further evaluation.

Recent and current explorations in the high and low mountains of the Western Ghats of India have led to the discovery of several unique species of the genus *Cnemaspis*. Although most of them are from isolated humid forest (Giri et al. 2009b; Srinivasulu et al. 2015; Cyriac et al. 2018; Khandekar et al. 2019a; Sayyed et al. 2019), ongoing studies are showing that *Cnemaspis* can also be found in drier regions. With the discovery of *C. palakkadensis* **sp. nov.**, the number of *Cnemaspis* species in the Indian mainland increases to 43, yet the true diversity within this group is clearly far from being totally uncovered. Recent studies have also hinted at the presence of cryptic diversity within the south Asian *Cnemaspis* (Agarwal et al. 2017; Cyriac et al. 2020). The current study further calls attention to cryptic diversity within the Western Ghats and adjacent low-lying regions. Thus, widespread fine-scale sampling will be critical for uncovering species richness and distributional patterns within the group.

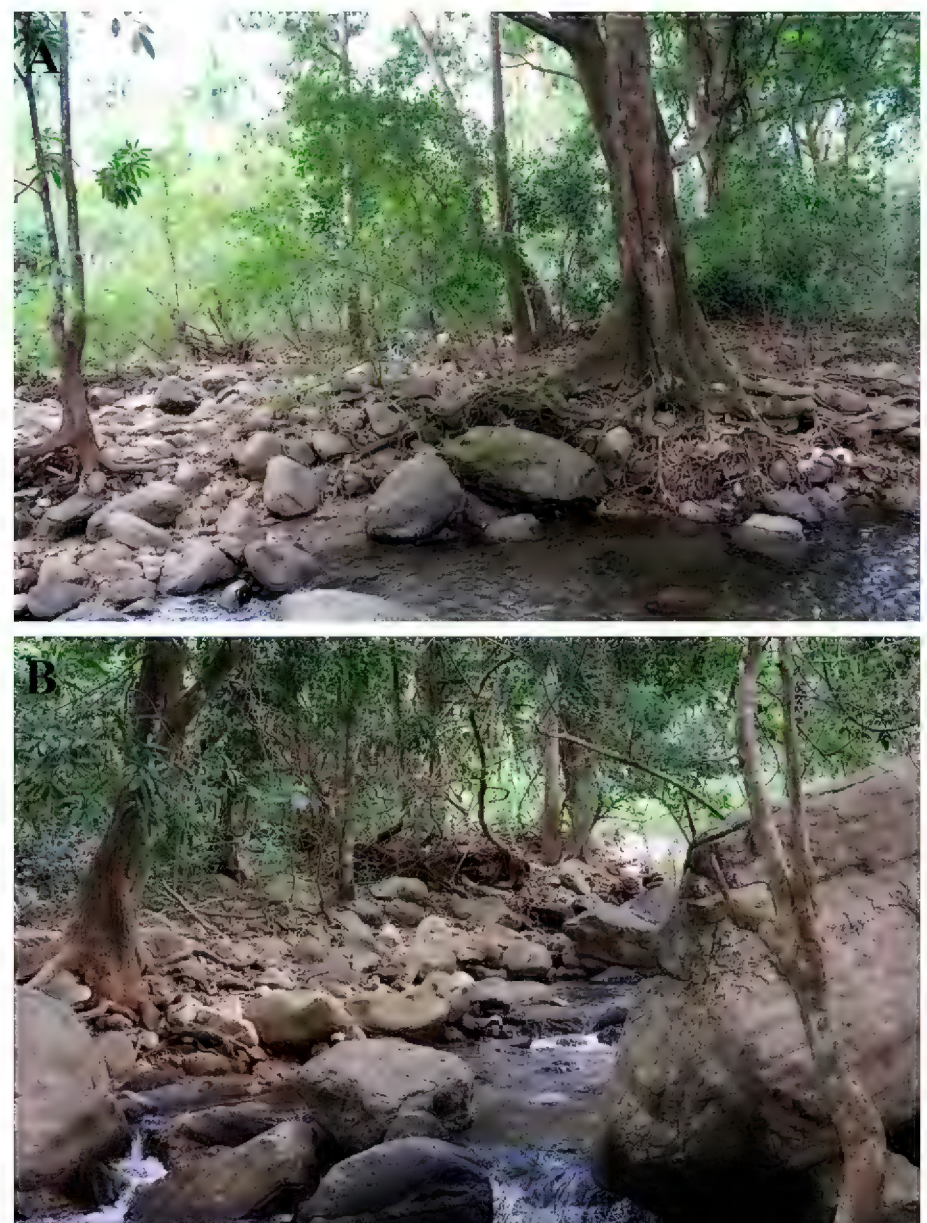


Fig. 8. Habitat of *Cnemaspis palakkadensis* **sp. nov.** Photos by Amit Sayyed.

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Literature Cited

- Bauer AM. 2002. Two new species of *Cnemaspis* (Reptilia: Squamata: Gekkonidae) from Gund, Uttara Kannada, India. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* 99: 155–167.
- Biju SD, Garg S, Gururaja KV, Shouche Y, Walujkar SA. 2014. DNA barcoding reveals unprecedented diversity in Dancing Frogs of India (Micrixalidae, *Micrixalus*): a taxonomic revision with description of 14 new species. *Ceylon Journal of Science (Biological Sciences)* 43(1): 37–123.
- Chaitanya R, Giri VB, Deepak V, Datta-Roy A, Murthy BHCK, Karanth P. 2019. Diversification in the mountains: a generic reappraisal of the Western Ghats endemic gecko genus *Dravidogecko* Smith, 1933 (Squamata: Gekkonidae) with descriptions of six new species. *Zootaxa* 4688: 1–56.
- Cyriac VP, Umesh PK. 2013. Current status of *Cnemaspis littoralis* (Jerdon, 1853) (Sauria: Gekkonidae) with designation of a neotype. *Taprobanica: the Journal of Asian Biodiversity* 5: 36–43.
- Cyriac VP, Umesh PK. 2014. Description of a new ground-dwelling *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae), from Kerala, allied to *C. wynadensis* (Beddome, 1870). *Russian Journal of Herpetology* 21(3): 187–194.
- Cyriac VP, Kodandaramaiah U. 2017. Paleoclimate determines diversification patterns in the fossorial snake family Uropeltidae Cuvier, 1829. *Molecular Phylogenetics and Evolution* 116: 97–107.
- Cyriac VP, Johny A, Umesh PK, Palot MJ. 2018. Description of two new species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the Western Ghats of Kerala, India. *Zootaxa* 4459: 85–100.
- Cyriac VP, Palot MJ, Deuti K, Umesh PK. 2020. A preliminary 16S rRNA phylogeny of the Indian *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) with the description of two new cryptic species from the *C. wynadensis* clade. *Vertebrate Zoology* 70(2): 171–193.
- Das I, Bauer AM. 2000. Two new species of *Cnemaspis* (Sauria: Gekkonidae) from Tamil Nadu, southern India. *Russian Journal of Herpetology* 7(1): 17–28.
- Das I, Sengupta S. 2000. A new species of *Cnemaspis* (Sauria: Gekkonidae) from Assam, northeastern India. *Journal of South Asian Natural History* 5(1): 17–23.
- Gamble T, Greenbaum E, Jackman TR, Russell AP, Bauer AM. 2012. Repeated origin and loss of adhesive toepads in geckos. *PLoS ONE* 7(6): e39429.
- Giri VB, Agarwal I, Bauer AM. 2009a. Designation of a neotype for *Cnemaspis mysoriensis* (Jerdon 1853) (Sauria: Gekkonidae), with a re-description and notes on its distribution and habitat. *Russian Journal of Herpetology* 16: 256–264.
- Giri VB, Bauer AM, Gaikwad KS. 2009b. A new ground-dwelling species of *Cnemaspis* Strauch (Squamata: Gekkonidae) from the northern Western Ghats, Maharashtra, India. *Zootaxa* 2164: 49–60.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780.
- Khandekar A. 2019. A new species of rock-dwelling *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from Tamil Nadu, southern India. *Zootaxa* 4571: 383–397.
- Khandekar A, Thackery T, Agarwal I. 2019a. Two more new species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the northern Western Ghats, Maharashtra, India. *Zootaxa* 4656: 43–70.
- Khandekar A, Gaitonde N, Agarwal I. 2019b. Two new *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the Shevaroy massif, Tamil Nadu, India, with a preliminary ND2 phylogeny of Indian *Cnemaspis*. *Zootaxa* 4609: 68–100.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1,547–1,549.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34(3): 772–773.
- Manamendra-Arachchi K, Batuwita S, Pethiyagoda R. 2007. A taxonomic revision of the Sri Lankan day-geckos (Reptilia: Gekkonidae: *Cnemaspis*), with descriptions of new species from Sri Lanka and southern India. *Zeylanica* 7: 9–122.
- Mirza ZA. 2018. A new cryptic species of ground-dwelling *Hemidactylus* (Squamata: Gekkonidae) from southern India. *Phyllomedusa* 17: 169–180.
- Mirza ZA, Pal S, Bhosale HS, Sanap RV. 2014. A new species of gecko of the genus *Cnemaspis* Strauch, 1887 from the Western Ghats, India. *Zootaxa* 3815: 494–506.
- Mukherjee D, Bhupathy S, Nixon AMA. 2005. A new species of day gecko (Squamata, Gekkonidae, *Cnemaspis*) from the Anaikatti Hills, Western Ghats, Tamil Nadu, India. *Current Science* 89: 1,326–1,328.
- Murthy BHC, Nitesh A, Sengupta S, Deepak P. 2019. A new species of day gecko of the genus *Cnemaspis*

- Strauch, 1887 (Squamata: Gekkonidae) from the Nilgiri Hills, Tamil Nadu, India. *Records of the Zoological Survey of India* 119(3): 211–226.
- Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274.
- Palumbi SR. 1996. PCR and molecular systematics. *Molecular Systematics* 2: 205–247.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The Simple Fool's Guide to PCR, Version 2.0*. University of Hawaii, Honolulu, Hawaii, USA. 45 p.
- Pyron RA, Burbrink FT, Wiens JJ. 2013. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. *BMC Evolutionary Biology* 13(1): 1–54.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robin VV, Sinha A, Ramakrishnan U. 2010. Ancient geographical gaps and paleo-climate shape the phylogeography of an endemic bird in the sky islands of southern India. *PLoS ONE* 5(10): e13321.
- Sayyed A, Pyron RA, Dahanukar N. 2016. *Cnemaspis flaviventralis*, a new species of gecko (Squamata: Gekkonidae) from the Western Ghats of Maharashtra, India. *Journal of Threatened Taxa* 8: 9,619–9,629.
- Sayyed A, Pyron RA, Dileepkumar R. 2018. Four new species of the genus *Cnemaspis* Strauch, 1887 (Sauria: Gekkonidae) from the northern Western Ghats, India. *Amphibian & Reptile Conservation* 12(2) [General Section]: 1–29 (e157).
- Sayyed A, Grismer LL, Campbell PD, Dileepkumar R. 2019. Description of a cryptic new species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the Western Ghats of Kerala State of India. *Zootaxa* 4656: 501–514.
- Shanker K, Vijayakumar SP, Ganeshaiah KN. 2017. Unpacking the species conundrum: philosophy, practice, and a way forward. *Journal of Genetics* 96(3): 413–430.
- Srinivasulu C, Kumar GC, Srinivasulu B. 2015. A new species of *Cnemaspis* (Sauria: Gekkonidae) from Northern Karnataka, India. *Zootaxa* 3947: 85–98.
- Uetz P, Hošek J. 2019. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 5 January 2020].
- Van Bocxlaer I, Biju SD, Willaert B, Giri VB, Shouche YS, Bossuyt F. 2012. Mountain-associated clade endemism in an ancient frog family (Nyctibatrachidae) on the Indian subcontinent. *Molecular Phylogenetics and Evolution* 62(3): 839–847.
- Vijayakumar SP, Dinesh KP, Prabhu MV, Shanker K. 2014. Lineage delimitation and description of nine new species of bush frogs (Anura: *Raorchestes*, Rhacophoridae) from the Western Ghats Escarpment. *Zootaxa* 3893(4): 451–488.
- Vijayakumar SP, Menezes RC, Jayarajan A, Shanker K. 2016. Glaciations, gradients, and geography: multiple drivers of diversification of bush frogs in the Western Ghats Escarpment. *Proceedings of the Royal Society B: Biological Sciences* 283(1836): 20161011.
- Zachariah A, Dinesh KP, Kunhikrishnan E, Das S, Raju DV, Radhakrishnan C, Palot MJ, Kalesh S. 2011. Nine new species of frogs of the genus *Raorchestes* (Amphibia: Anura: Rhacophoridae) from southern Western Ghats, India. *Biosystematica* 5: 25–48.
- Zachariah A, Cyriac VP, Chandramohan B, Ansil BR, Mathew JK, Raju DV, Abraham RK. 2016. Two new species of *Raorchestes* (Anura: Rhacophoridae) from the Silent Valley National Park in the Nilgiri Hills of the Western Ghats, India. *Salamandra* 52(2): 63–76.
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* 20(1): 348–355.
- Zheng Y, Wiens JJ. 2016. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4,162 species. *Molecular Phylogenetics and Evolution* 94: 537–547.



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Vivek Cyriac is an evolutionary ecologist from India with broad interests in the ecological and evolutionary mechanisms that generate biodiversity patterns. His work is centered around understanding how environmental factors and biotic interactions influence species diversification and create macro-evolutionary patterns. Vivek uses reptiles and amphibians as model systems to explore diverse questions in ecology, evolution, and behavior. Thus far, he has predominantly worked on fossorial uropeltid snakes and geckos of the genus *Cnemaspis*.



Raveendan Dileepkumar is currently a Principal Investigator under the Young Investigator's Program in Biotechnology in the Centre for Venom Informatics, University of Kerala, India. He is also co-investigating projects in the area of venomomics supported by KSCSTE, Government of Kerala. His research broadly encompasses the venomomics, venom gland transcriptomics, and genomics of venomous snakes; with ongoing projects centered on understanding the venom composition of venomous species in the animal kingdom. His publications, including book chapters, have focused on snake taxonomy, venomomics, and venom applications in medical technologies.

A new *Cnemaspis* species from India

Appendix 1. GenBank accession number and voucher information for Indian *Cnemaspis* and outgroups used in the phylogenetic analysis. The line highlighted in bold indicates a new sequence generated for *C. palakkadensis* **sp. nov.** * indicates accession number of *C. kottiyooorensis* which was misprinted as MT217042 in Cyriac et al. (2020).

No	Species	Locality	Voucher	16s rRNA
1	<i>Cnemaspis mahabali</i>	Pune, Maharashtra	ZSI/R/1048	KX753643
2	<i>Cnemaspis amboliensis</i>	Sindhudurg, Maharashtra	BNHS 2458	MH174358
3	<i>Cnemaspis ajijae</i>	Satara, Maharashtra	ZSI WRC R/1058	KX753653
4	<i>Cnemaspis limayei</i>	Sindhudurg, Maharashtra	ZSI WRC R/1053	KX753647
5	<i>Cnemaspis yercaudensis</i>	Salem, Tamil Nadu	BNHS 2510	MH174360
6	<i>Cnemaspis otai</i>	Vellore, Tamil Nadu	BNHS 2512	MH174362
7	<i>Cnemaspis gracilis</i>	Palakkad, Kerala	BNHS 2514	MH174370
8	<i>Cnemaspis indica</i>	Nilgiris, Tamil Nadu	BNHS 2516	MH174366
9	<i>Cnemaspis littoralis</i>	Kozhikode, Kerala	BNHS 2517	MH174367
10	<i>Cnemaspis littoralis</i>	Kozhikode, Kerala	BNHS 2518	MH174368
11	<i>Cnemaspis kottiyooorensis</i>	Kannur, Kerala	BNHS 2519	MH174363
12	<i>Cnemaspis wynadensis</i>	Wayanad, Kerala	BNHS 2520	MH174364
13	<i>Cnemaspis goaensis</i>	Goa	ZSI WRC R/1045	KX269826
14	<i>Cnemaspis flaviventralis</i>	Sindhudurg, Maharashtra	ZSI WRC R/1043	KX269820
15	<i>Cnemaspis girii</i>	Satara, Maharashtra	BNHS 2446	KX269824
16	<i>Cnemaspis kolhapurensis</i>	Sindhudurg, Maharashtra	BNHS 2448	KX269822
17	<i>Cnemaspis heteropholis</i>	Shimoga, Karnataka	BNHS 2466	KX753660
18	<i>Cnemaspis adii</i>	Ballari, Karnataka	BNHS 2465	KX753655
19	<i>Cnemaspis goaensis</i>	Goa	ZSI WRC R/1044	KX269825
20	<i>Cnemaspis zacharyi</i>	Lakkadi, Wayanad, Kerala	BNHS 2735	MT217042
21	<i>Cnemaspis chengodumalaensis</i>	Chengodumala, Kozhikode, Kerala	BNHS 2741	MT217043
22	<i>Cnemaspis</i> sp. (Pepara)	Pepara WLS, Trivandrum, Kerala	VPCGK_014	MT217033
23	<i>Cnemaspis anamudiensis</i>	Anamudi RF, Idukki, Kerala	VPCGK_016	MT217034
24	<i>Cnemaspis</i> sp. (Vagamon)	Vagamon, Kerala	VPCGK_021	MT217035
25	<i>Cnemaspis heteropholis</i>	Devarakolli, Madikeri, Karnataka	BNHS 2745	MT217039
26	<i>Cnemaspis kottiyooorensis</i>	Devarakolli, Madikeri, Karnataka	BNHS 2747	MT217038
27	<i>Cnemaspis kottiyooorensis</i>	Paithalmala, Kannur, Kerala	VPCGK_052	MT217037*
28	<i>Cnemaspis palakkadensis</i> sp. nov.	Anakkal, Palakkad, Kerala	BNHS 2790	MT762366
Outgroups				
29	<i>Phelsuma lineata</i>	Madagascar	ZCMV_2029	KC438463
30	<i>Phelsuma v-nigra</i>	Moheli, Comoros	MH10	FJ829967
31	<i>Phelsuma ornata</i>	Reunion	Sound_P7	DQ270577
32	<i>Lygodactylus picturatus</i>	Tanzania	LYG_4	HQ872462
33	<i>Lygodactylus miops</i>	Madagascar	LUS8	LN998673
34	<i>Lygodactylus madagascariensis</i>	Madagascar	LM1A	LN998665

Appendix 2. Specimens examined.

Cnemaspis aaronbaueri: BNHS 2607, BNHS 2608, and BNHS 2609 (females), from Thenmala, Kollam District, Kerala, India.

Cnemaspis beddomei: collection of the Natural History Museum, London (NHMUK), NHMUK 1946.9.4.83 (male), from South Tinnevely, Tirunelveli, southern Tamil Nadu State, India.

Cnemaspis gracilis: NHMUK 74.4.29.393 (male), from “Palghat Hills” (Kerala State, India), and BNHS 2513 and BNHS 2514, collected from the Palakkad, Kerala, used for examination and genetic analysis.

Cnemaspis indica: NHMUK 46.11.22.22b (male), BNHS 1252–10 (male), Nilgiris, Tamil Nadu, India.

Cnemaspis kolhapurensis: BNHS 1855 (male), Dajipur, Kolhapur district, Maharashtra; and BNHS 2447 and BNHS 2448, from Amboli, Sindhudurg district, Maharashtra, India.

Cnemaspis kottiyorensis: BNHS 2519 from Kannur, Kerala state, India.

Cnemaspis littoralis: Neotype ZSI/WGRC/IR/V/2377 (male) from Chaliyam, Kozhikode, Kerala; ZSI/WGRC/IR/V/2378a and ZSI/WGRC/IR/V/2378b (males) from Narayamkulam (= Chengodumala), Kozhikode, Kerala; ZSI/WGRC/IR/V/2379a (male) and ZSI/WGRC/IR/V/2379b (female) from Kapprikad, Ernakulam, Kerala; ZSI/WGRC/IR/V/2380 (male) from Chaliyam, Kozhikode, Kerala; ZSI/WGRC/IR/V/2381a and ZSI/WGRC/IR/V/2381b (males) from Nellikuth, Mallapuram, Kerala; BNHS 1150 (male), from Nilambur, Malabar, Kerala state, India. BNHS 2517 and BNHS 2518 from the Kozhikode, Kerala state, India.

Cnemaspis maculicollis: ZSI/WGRC/IR/V/2704 (male), from Pandimotta, Shendurney Wildlife Sanctuary, Kollam District, Kerala, India.

Cnemaspis nilagirica: NHMUK 74.4.29.729 (female), Nilgiris, Nilgiri District, Tamil Nadu State, south-western India.

Cnemaspis ornata: Lectotype NHMUK 74.4.29.400 (male), paralectotype NHMUK 74.4.29.401 (male), NHMUK 74.4.29.404 (female), NHMUK 74.4.29.405 (female), NHMUK 74.4.29.406 (female), NHMUK 74.4.29.407 (female), NHMUK 74.4.29.408 (female), and NHMUK 74.4.29.409 (female), from South Tinnevely Hills, Tirunelveli, Tamil Nadu State, India.

Cnemaspis sisparensis: NHMUK 74.4.29.383 (male), from Sholakal, the foot of SisparaGhat, Kerala, India.

Cnemaspis wynadensis: BMNH 74.4.29.355 (male), from Wynaad, Kerala, and BNHS 1042, BNHS 1043 (male), Mannarghat, Palghat, Kerala, India.



Amphibian diversity and conservation along an elevational gradient on Mount Emei, southwestern China

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Abstract.—Understanding the diversity, distribution, and threat status of species serves an important role in biodiversity conservation, particularly in regions with high species richness. Being a well-known Natural and Cultural World Heritage site, Mount Emei is seated on the transition zone between Qinghai-Tibetan Plateau and Sichuan Basin in southwestern China, and is of special significance to conservation and science due to its high biodiversity. Based on data from extensive field expeditions, the published literature, and museum specimens, this study documented a total of 35 species, belonging to 22 genera and nine families, along a 2,600 m elevational gradient on Mount Emei. Almost one-third of these species are in IUCN threatened categories. A majority of species occupied a narrower local elevation range size compared with their overall elevation range size, especially those that are threatened. Along the elevational gradient, both the total and threatened species richness showed hump-shaped patterns. These results provide insight into the species diversity, elevational distribution, and threat status for the amphibians on Mount Emei. These findings highlight the significance and urgent need to protect the amphibians in the focal region, provide support for further ecological studies, and will contribute to the conservation of this biodiverse region in the future.

Keywords. Anura, biodiversity, Caudata, hump-shaped pattern, species richness, threatened species, World Heritage site

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Introduction

Elevational gradients provide one of the most powerful natural experiments for exploring the ecological and evolutionary responses of biota to the complex influences of geophysical and climatic changes (Korner 2007). In mountain regions, elevational gradients yield a large amount of environmental variation (e.g., in temperature and humidity) over a short spatial distance, and play a prominent role in shaping vertical species distribution (Korner 2007; Perrigo et al. 2019). Following in von Humboldt's footsteps, the importance of elevational gradients to biodiversity has motivated growing scientific interest in them over the last two centuries (Aynekulu et al. 2012; Frishkoff et al. 2019; Lomolino 2001). Along elevational gradients, a multitude of studies have focused on species diversity-elevation relationships across many different taxa worldwide (e.g., Frishkoff et al. 2019; Hu et al. 2011; Longino and Branstetter 2019; Peters et al. 2016; Quintero and Jetz 2018), often revealing monotonic decreasing, hump-

shaped patterns or plateaus (Rahbek 2005). While varying degrees of evidence have supported different patterns, understanding the elevational patterns in biodiversity remains crucial for conservation in specific key biodiversity areas and in some taxa which are not well documented.

Among amphibians, the distribution range of a species is highly related to its adaptation to environmental variations and extinction risk (Chen et al. 2019; Cooper et al. 2008). Amphibians with a small geographic (e.g., latitudinal or elevational) range size may face higher extinction risk, since they are relatively less abundant, less mobile, and more easily influenced by local environment changes, compared with those with broad ranges (Botts et al. 2013; Chen et al. 2019; Cooper et al. 2008). Since each species has a unique ecological extension and environmental tolerance (Wells 2007), range size and its shifts along elevational gradients can be regarded as adaptive responses to environmental changes (Chen et al. 2009; Kusrini et al. 2017). Consequently, determining elevational range size

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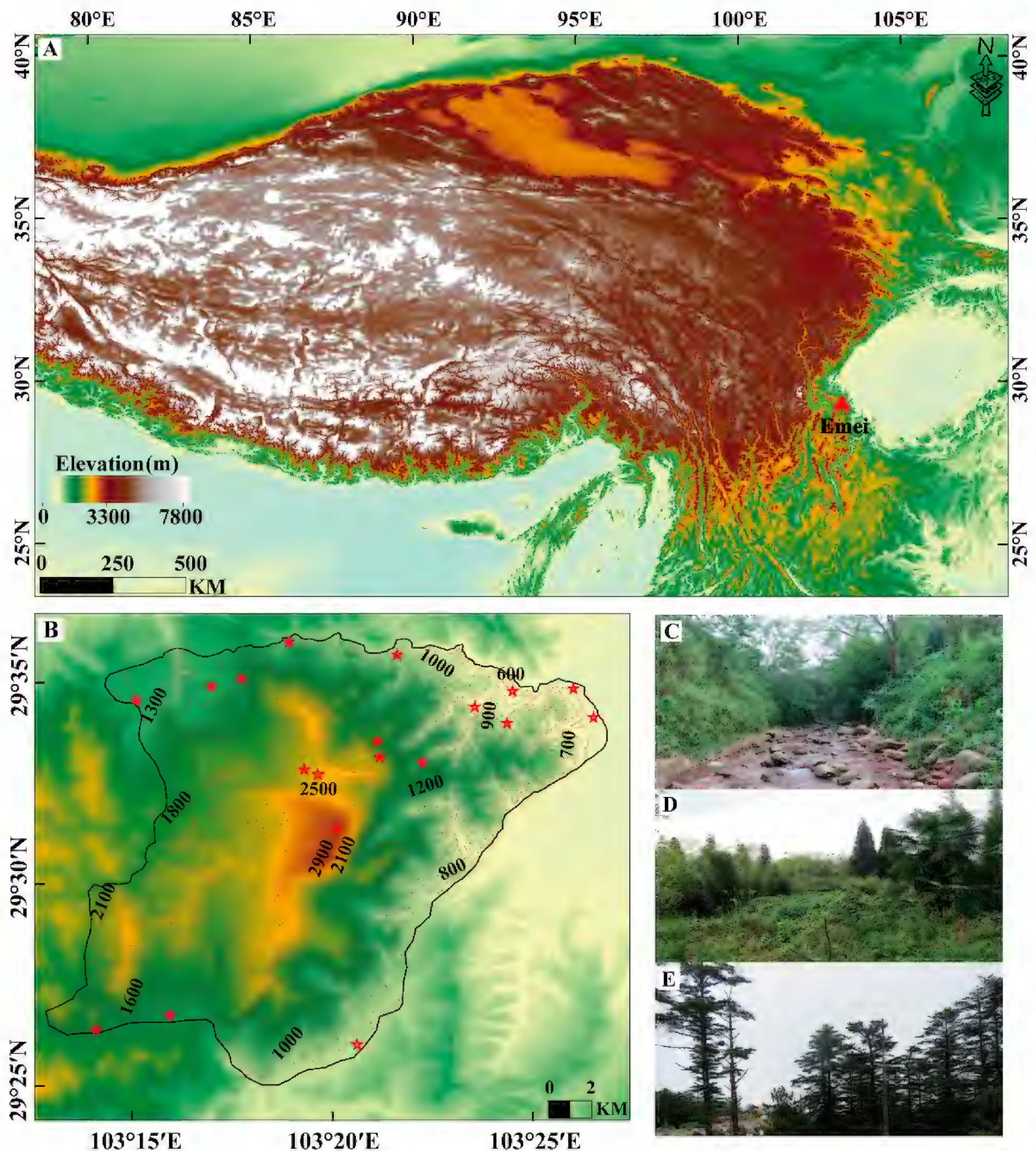


Fig. 1. (A) Geographic location of Mount Emei; (B) topographic overview of sample sites; and dominant vegetation types and typical habitats along the elevational gradient at (C) 500 m, (D) 1,300 m, and (E) 3,050 m. Sampling sites are indicated with red stars (see Appendix 1 for details).

under various environmental conditions is important for contributing to the conservation of amphibians with a narrow range (Chan et al. 2016).

Mount Emei (Omei Shan) is located in the transitional zone between the Sichuan Basin and Qinghai-Tibetan Plateau in southwestern China (Fig. 1A). It possesses various landscapes and a diversity of natural biological zones with high biodiversity (Li and Shi 2007; Zhao and Chen 1980). Mount Emei, together with the Leshan Giant

Buddha Scenic Area, constitutes a Natural and Cultural World Heritage site due to the striking scenic beauty, and its exceptional spiritual and cultural significance in Chinese Buddhism (<http://whc.unesco.org/en/list/779>). In addition, the high biodiversity highlights its special significance to conservation and science (Zhao and Chen 1980). For amphibians, increasing attention has been paid to this region in the past several decades. Liu (1950) had conducted field surveys since 1938, and Fei et al.

(1976) roughly delineated the elevational distributions for 32 species. However, there were no scientific reports concerning amphibians on Mount Emei for nearly 40 years after these early studies, except for some scattered sightings and samplings. By recording 24 species, Zhao et al. (2018) recently documented the idiosyncratic contributions of individual species to the taxonomic, functional, and phylogenetic diversity on Mount Emei. While these studies presented different facets of diversity, efforts to provide a comprehensive inventory by integrating amphibian species diversity, distribution, and threat status on Mount Emei have remained severely limited.

This study aims to summarize and update data on amphibian species richness on Mount Emei, and also to delineate the distribution and threat status of each species based on extensive field expeditions, supplemented with data from the literature (Fei et al. 1976; Liu 1950; Zhao et al. 2018) and specimen records in the Herpetological Museum, Chengdu Institute of Biology (CIB), Chinese Academy of Sciences (CAS). This study will be helpful in the development of effective conservation strategies for the amphibians on Mount Emei and the surrounding areas.

Materials and Methods

Study Area

Mount Emei, a mountain in southwestern China which is known worldwide, was formed on the southwestern edge of Sichuan Basin, China, and dates from the late Cretaceous Period around 70 million years ago (Zhao and Chen 1980). From the base at about 500 m asl, the mountain rises to an altitude of 3,099 m (Fig. 1B). It is made up of deep canyons and narrow gorges (Tang 2006), which results in a variety of attractive landscapes and complex environments to support exceptionally rich flora and fauna (Li and Shi 2007; Zhao and Chen 1980). Mount Emei is characterized by a subtropical monsoon climate. The annual average temperature drops from 17 °C to 3 °C with the increasing elevation. The rainfall is abundant and concentrated during May–September, without a dry season (Tang 2006), and the highest rainfall occurs in the middle and high mountain areas (Li 1990).

The parent rocks in this region mainly include shale, dolomite, limestone, basalt, sandstone, and mudstone (Zhao and Chen 1980); and the following natural vertical soil zones have been described: yellow soil and mountain yellow soil sandwich a purple soil zone (below 1,800 m), mountain yellow-brown soil zone (1,800–2,200 m), mountain dark brown soil zone (2,200–2,600 m), and podzolic soil and meadow soil zone (above 2,600 m) [Li 1990; Tang 2006]. Additionally, Mount Emei is situated at the junction between the tropical and temperate zonation types in eastern Asia (Tang and Ohsawa 1997). There are three major vegetation types along the elevational gradient (from low to high): evergreen broad-

leaved forest, evergreen deciduous broad-leaved mixed forest, and coniferous forest (Li and Shi 2007; Tang and Ohsawa 1997) [Fig. 1C–E].

Species Data

The field surveys included 23 line transects and three sampling points along the elevational gradient to comprehensively investigate the amphibian species composition on Mount Emei during the breeding seasons in 2017 and 2018 (Fig. 1B; Appendix 1). During the field surveys, line transects and sampling points were mainly placed near water resources according to habitat conditions, and locations were recorded by a global positioning system (GPS) app (Shenzhen 2bulu Information Technology Company). Observers (at least two persons) intensively searched for amphibians with an electric torch and searched systematically at a relatively steady pace (about 2.0 km h⁻¹) at night (1900–2400 h), with the locations of observed individuals being recorded by the GPS.

Complementary information was also collected from the literature (Fei et al. 1976; Liu 1950; Zhao et al. 2018), with useful information extracted on the taxonomy, species composition, and elevational distribution of amphibians. Species data were also supplemented with records from museum specimens in the CIB/CAS. The preserved specimen and recorded information were carefully authenticated and crosschecked, and records possibly representing missing species detections and/or misidentifications during sampling or secondary information compilation were removed. In total, there were 35 amphibian species scientifically recorded, with available elevation information for 34 of the species (all except *Amolops granulosus*).

Data Compilation and Analysis

Species nomenclature followed *Amphibian Species of the World* (Frost 2019). Referring to both the IUCN Red List (IUCN 2018) and the China Biodiversity Red List (MEP and CAS 2015), the threat status levels for each species were compared at the global and national scales. A database was generated with the species components, elevational distribution data (minimal and maximal elevations of occurrence), and threat status of each species.

The overall elevational range spanning 500–3,099 m was divided into 200 m band widths, and areas with elevation ranges between 500–1,299 m were defined as low elevations, ranges between 1,300–2,099 m as middle elevations, and ranges between 2,100–3,099 m as high elevations. For each species, the elevational distribution was assumed to cover a continuous range between the minimum and maximum documented elevations (Hu et al. 2011; Rahbek 1997). For example, a species with recorded elevation limits between 1,235 and 1,450 m can be classified into both the 1,100–1,299 m and 1,300–

1,499 m bands. Species richness was calculated from the total (cumulative) number in each band and each species' threat status was assessed for the different bands. In addition, three polynomial regressions (richness as a function of elevation, elevation², and elevation³) were used to investigate the richness-elevation relationships for total species and threatened species (at both global and national scales) based on the smallest corrected Akaike information criterion (AICc) value.

Next, the overall range size (i.e., elevational range covering the whole distribution range of a species) was collected from the literature (Fei et al. 2006, 2009a,b, 2012) and the online database (*Amphibian Species of the World*, Frost 2019). Local elevational range size (maximal minus minimal elevation) observed on Mount Emei was plotted and compared with the overall range size for each species. The elevational range size values below the median value (i.e., 1,300 m) were considered “small,” and they were considered “large” for those that were not less than the median.

Results

The 35 species belonged to 22 genera and nine families. Of special note, Mount Emei was the type locality of 14 species, including one endemic species (*Rana chevronata*; Table 1). At the family level, Megophryidae and Ranidae were the two most abundant families (each with 11 species) and they contributed approximately 63% of all amphibian species on this mountain; followed by Rhacophoridae (four species); Microhylidae, Dicroglossidae, and Hynobiidae (each with two species); and the other three families (Bufonidae, Hylidae, and Cryptobranchidae) were each represented by a single species (Table 1). According to the IUCN Red List, 13 of the 35 species were categorized as threatened, including two Critically Endangered (CR), five Endangered (EN), three Vulnerable (VU), and three Near Threatened (NT) species; while according to the China Biodiversity Red List, 16 of the 35 species were categorized as threatened, including one CR, three EN, nine VU, and three NT species (Table 1).

Along the elevational gradients, a cubic relationship was statistically favored over either a quadratic or linear relationship for the total species richness, while a quadratic relationship was favored over cubic or linear for the threatened species richness at the two scales (Fig. 2; Table 2). Both species richness and threatened species richness showed mid-elevation peak patterns (Fig. 2). Low and middle elevations (500–2,099 m) were found to harbor a majority of species with a maximum richness at the two elevational bands of 700–899 m and 1,500–1,699 m (Fig. 2). There was a sharp decrease between 1,900–2,099 m and 2,100–2,299 m, while species richness changed only slightly for elevations above 2,100 m (Fig. 2). Similar patterns were found for the threatened species, with a maximum in the band at 1,700–1,899 m (Fig. 2;

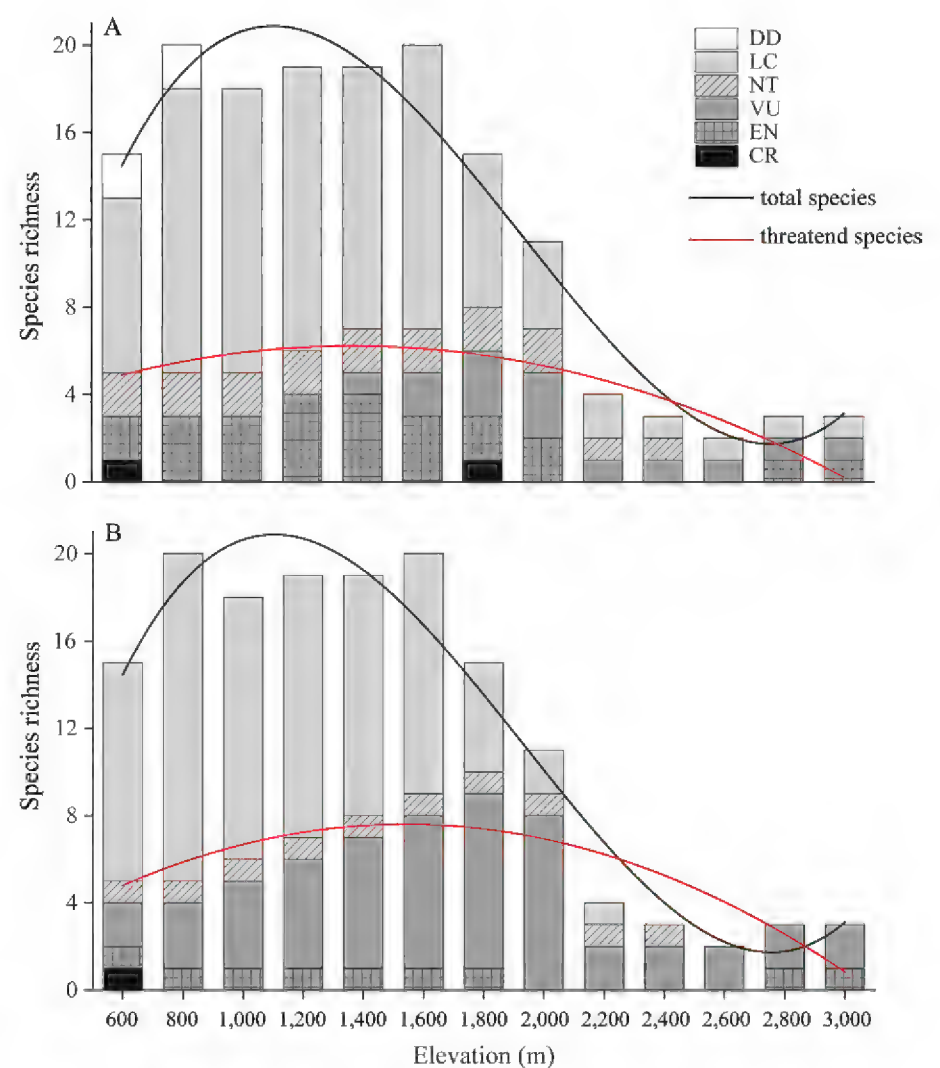


Fig. 2. The numbers of total and threatened species (bars) and elevational patterns of species richness (curves). Regression lines show total species richness (black) and threatened species richness (red) based on the polynomial regression models, with threatened status counts referring to the IUCN Red List (A) and the China Biodiversity Red List (B).

Table 1). Referring to the IUCN Red List, 12 threatened species occurred in low and middle elevations, while three threatened species occurred in high elevations (Fig. 2A; Table 1); referring to the China Biodiversity Red List, 14 threatened species were in low and middle elevations, and four threatened species were in high elevations (Fig. 2B; Table 1).

Although the upper elevational limits of three species (i.e., *Scutiger chintingensis*, *Batrachuperus pinchonii*, and *Rhacophorus dugritei*) were higher than 3,000 m, and four species (i.e., *Xenophrys omeimontis*, *X. minor*, *Oreolalax omeimontis*, and *O. major*) were found to exceed the overall range size, 26 of the species have a small range size (< 1,300 m) on Mount Emei (Fig. 3; Table 1). In total, ten of the threatened species have a small range size on Mount Emei (Table 1). Notably, the Critically Endangered species (*Andrias davidianus*) and the endemic (*R. chevronata*) were each restricted to an extremely narrow range. The local range size was relatively wider than the overall range size for some of the threatened species (e.g., *B. londongensis*, *R. chevronata*, *O. omeimontis*), but the range size of these species were reasonably small (Fig. 3; Table 1).

Discussion

Knowing where the individual species occur and identifying which ones are threatened and their

Amphibians on Mount Emei, China

Table 1. Amphibian species on Mount Emei, China, with their elevational distribution (minimal and maximal elevations of occurrence) and threat status.

Species	IUCN Red List	China Red List	Lower limit (m)	Upper limit (m)
I. Hynobiidae				
<i>Batrachuperus londongensis</i> ¹	EN	VU	1,200	1,400
<i>Batrachuperus pinchonii</i>	VU	VU	1,400	3,050
II. Cryptobranchidae				
<i>Andrias davidianus</i>	CR	CR	—	500
III. Megophryidae				
<i>Oreolalax major</i> ¹	VU	VU	1,500	2,000
<i>Oreolalax schmidtii</i> ¹	NT	NT	1,580	2,340
<i>Oreolalax multipunctatus</i> ¹	VU	VU	1,800	1,920
<i>Oreolalax omeimontis</i> ¹	EN	VU	740	2,060
<i>Oreolalax popei</i>	LC	VU	950	2,010
<i>Scutiger chintingensis</i> ¹	EN	EN	2,890	3,050
<i>Leptobrachium boringii</i> ¹	EN	EN	650	1,650
<i>Leptobrachella oshanensis</i> ¹	LC	LC	760	1,810
<i>Atympanophrys shapingensis</i>	LC	LC	—	2,120
<i>Xenophrys omeimontis</i> ¹	NT	VU	610	1,920
<i>Xenophrys minor</i>	LC	LC	680	1,600
IV. Bufonidae				
<i>Bufo gargarizans</i>	LC	LC	500	1,910
V. Hylidae				
<i>Hyla annectans</i>	LC	LC	1,200	1,298
VI. Ranidae				
<i>Rana chevronta</i> ^{1,2}	CR	EN	1,750	1,850
<i>Rana omeimontis</i> ¹	LC	LC	500	2,080
<i>Pelophylax nigromaculatus</i>	NT	NT	500	1,300
<i>Boulengerana guentheri</i>	LC	LC	—	500
<i>Nidirana daunchina</i> ¹	LC	LC	750	1,660
<i>Odorrana graminea</i>	DD	LC	530	710
<i>Odorrana schmackeri</i>	LC	LC	530	790
<i>Odorrana margaretae</i>	LC	LC	500	1,810
<i>Amolops chunganensis</i>	LC	LC	720	1,600
<i>Amolops granulosus</i>	LC	NT	—	—
<i>Amolops mantzorum</i>	LC	LC	800	1,660
VII. Dicroglossidae				
<i>Quasipaa boulengeri</i>	EN	VU	500	1,900
<i>Fejervarya multistriata</i>	DD	LC	500	850
VIII. Rhacophoridae				
<i>Polypedates megacephalus</i>	LC	LC	740	1,600
<i>Rhacophorus chenfui</i> ¹	LC	LC	800	1,660
<i>Rhacophorus omeimontis</i> ¹	LC	LC	680	1,810
<i>Rhacophorus dugritei</i>	LC	VU	1,520	3,050
IX. Microhylidae				
<i>Microhyla fissipes</i>	LC	LC	500	530
<i>Kaloula rugifera</i>	LC	LC	700	900

¹Species type locality is Mount Emei; ²endemic species on Mount Emei. The threat status abbreviations refer to the IUCN Red List of Threatened Species and the China Biodiversity Red List: Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR).

Table 2. Results of polynomial regression models for assessing the total species and threatened species patterns along the elevational gradient.

Polynomial regressions	Total species richness	Threatened species (IUCN)	Threatened species (China)
First-order R ²	0.76***	0.22	0.39*
AICc	78.63	67.69	61.27
Second-order R ²	0.81***	0.57*	0.62**
AICc	79.90	64.20	59.47
Third-order R ²	0.95***	0.66*	0.70**
AICc	68.64	66.66	61.88

Tested effects were significant at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Bold numbers indicate the models which best accounted for variation in the richness along the elevation gradient based on the smallest AICc value.

conservation status are critical for optimizing the conservation of species and communities in a given region. This study documented the species component, distribution, and threat status of amphibians along a 2,600 m elevational gradient on Mount Emei in China. Although Mount Emei is a Natural and Cultural World Heritage site with high richness in amphibians, their threat status is really severe overall, particularly since a majority of the species possess relatively narrow local range sizes. Taken together, these results can contribute to a better understanding and more effective conservation of the amphibian diversity on this mountain.

The higher amphibian diversity on Mount Emei documented in this study, relative to the published records in this region (Fei et al. 1976; Liu 1950; Zhao et al. 2018) and the neighboring regions (e.g., Mount Gongga and Mount Erlang; Xie et al. 2007), underlines its great significance in conservation and scientific studies. Megophryidae and Ranidae are the two most species-rich families, accounting for 63% of all species in the region (Table 1); and the extremely adaptable capacities and enhanced environmental tolerances of some species may contribute to the dominance of these two families (Fei et al., 2009a,b; Wells 2007). At the species level, the endemic species (*R. chevronta*) with a narrowly specified range is actually rare and threatened, and it should be urgently targeted for conservation (Hu et al. 2012). Although the data in this study were obtained from extensive field surveys combined with comprehensive data collection, the results provided here may be missing certain information. Anecdotal observations of *Odorrana hejiangensis* on Mount Emei have been reported (K. Jiang, pers. comm.), but they were not verified from any scientific publication or field expedition. Therefore, further surveys in the region are still necessary.

Elevation is often regarded as a surrogate for temperature and moisture, and is widely used to investigate distributional patterns of species richness in mountainous regions (Khatriwada et al. 2019; Peters et al. 2016; Rahbek 1995). Variations of climatic variables, land surface area, and geography along elevational gradients are among the hypothesized causal factors influencing species composition and distribution (Lomolino 2001). Amphibians, which tend to have

complex life histories and relatively low mobility, are strongly restricted by the external environment (Hof et al. 2011; Wake and Vredenburg 2008). Climatic factors (mainly temperature and precipitation) are widely recognized as the key determinants that influence various aspects of amphibian biology, such as physiology, behavior, and ecological performance (Wells 2007). A habitat with a lower temperature and higher elevation is prone to support more species (Navas et al. 2013), and more abundant precipitation can support higher species richness and abundance (Rahbek 2005; Wells 2007). Under the influences of climatic, edaphic, and vegetation zones (Tang 2006; Tang and Ohsawa 1997), the vertical distribution pattern of amphibians on Mount Emei is obvious (Fig. 2). Indeed, low and middle elevations with higher temperatures (Tang 2006) and rainfall (Li 1990) are so suitable for amphibians that they support more species (Fig. 2). Additionally, it is well known that conserving a large number of species can provide the opportunity to conserve rare species and other undetected species (Aynekulu et al. 2012). That is, more conservation investment in the areas below 2,100 m on this mountain is needed because most of the amphibians and threatened species are restricted to the elevations below 2,100 m (Fig. 2). Even so, several species in high elevations (2,100–3,099 m) should also attract a great deal of attention because they can be considered as the indicators of environmental adaptation in the high elevations.

Range size is a critical factor that reflects the local assemblage structure (Gaston 1996) and a species' environmental niche (Pearson et al. 2006). It is recognized that species with different range sizes should be conserved with different strategies (Chen et al. 2019; Di Marco and Santini 2015). A small range size may be one of the strongest predictors of extinction risk (Chen et al. 2019; Rosenzweig 1995). In this study, some species have a larger local range size compared with the overall elevational range size but most species have a smaller local range size, especially among the threatened species (Fig. 3; Table 1). For instance, the range size is extremely narrow for *A. davidianus* (CR). Therefore, conservation priority should be given to these species with small range sizes (Chen et al. 2019). Range size can be influenced

Amphibians on Mount Emei, China

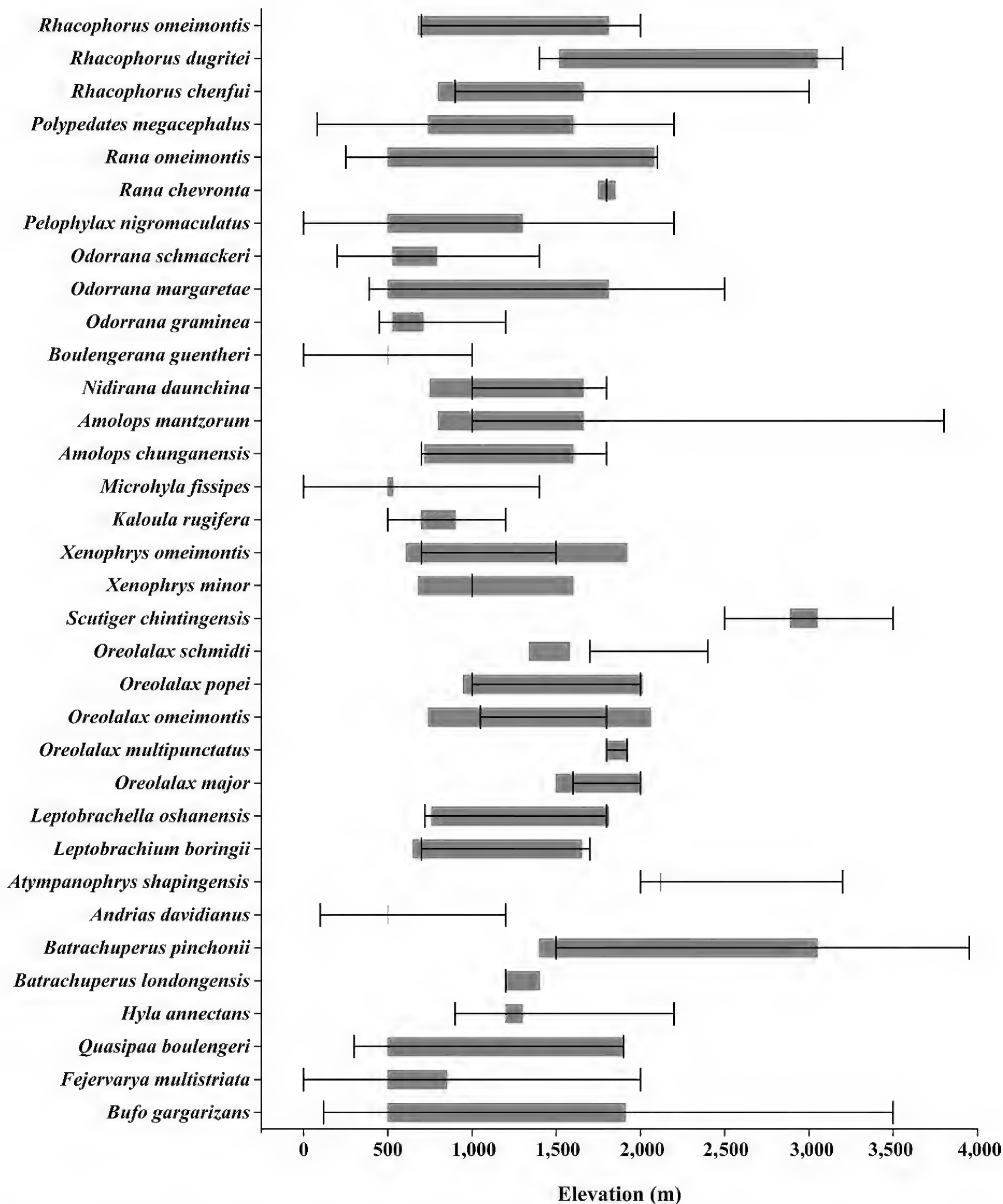


Fig. 3. Local and overall elevational ranges for each amphibian species. For each species, the local elevational range is the maximum minus minimum elevation on Mount Emei (gray box or vertical line), and the overall elevational range size is the published elevational range covering the whole distribution range (the horizontal line).

by environmental modification, as well as life-history and evolutionary traits (Gaston 1996). For example, increasing human activities and climate changes may lead to a range shift along the elevation (Chen et al. 2009; Kusrini et al. 2017). As a famous tourist attraction, the tourist season on Mount Emei overlaps with the breeding season of most amphibians, resulting in changes of the species' range size (Fei et al. 2009a,b; Liu and Yang

2012). On the other hand, individual intrinsic traits, such as dispersal abilities, habitat selection, and environmental tolerance, may indirectly contribute to a range shift under environmental changes (Fei et al. 2006, 2009a,b; Gaston 1996). For example, tadpoles and some stream-dwelling adults may be flushed downstream in running water, leading to a lower minimal elevation. Of course, one caveat must be applied to the results. Although

this survey illustrated that a species' range size may be related to its threat status, it did not examine the extent of that correlation based on any statistical support. There is a need to further explore the influences of intrinsic traits (e.g., range size, body size, and clutch size) on extinction risk with more detailed data.

Amphibians are a major group that is currently at risk globally (Jiang et al. 2016; Wake and Vredenburg 2008), with declines which far exceed those of other vertebrate taxa (Hoffmann et al. 2010). Accumulating evidence indicates that amphibians are threatened by anthropogenic land-use changes, fatal chytridiomycosis, climatic changes, and over-exploitation (Blaustein and Kiesecker 2002; Hof et al. 2011). Mount Emei suffers from intense human disturbance (e.g., cultivation and tourism), and nearly one-third of the amphibian species are severely at risk as indicated by their currently threatened status (Table 1). As such, urgent conservation actions are necessary for amphibians. Although biodiversity conservation and environmental management awareness among local governments and the public have been strengthened, the conflict between conservation and socioeconomic development continues to make biodiversity conservation exceptionally difficult to achieve. In this context, understanding how species respond to human-disturbances and survive in the human-dominated landscape is critical to the conservation of amphibians in mountain systems. This study can be helpful for scientifically-based policy making and for implementing the regulatory measures to mitigate the potential disturbances on biodiversity caused by mass-tourism.

Conclusion

In summary, this study presents data on the species richness, distribution, and threat status for 35 amphibian species in a tourist attraction, Mount Emei in China, which is a site of special significance to conservation and to science. The results highlight the urgent need to manage and preserve the amphibians, especially the threatened species, and will be helpful in assisting with sustainable management and the development of effective conservation strategies. These findings can also provide a basis for further ecological studies, such as exploring intraspecific and/or interspecific responses to biotic and abiotic influences (Hu et al. 2019; Huang et al. 2020; Wang et al. 2019), not only for the focal mountain but also for other similar regions or high-profile areas of concern.

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Literature Cited

- Aynekulu E, Aerts R, Moonen P, Denich M, Gebrehiwot K, Vågen T-G, Mekuria W, Boehmer HJ. 2012. Altitudinal variation and conservation priorities of vegetation along the Great Rift Valley escarpment, northern Ethiopia. *Biodiversity and Conservation* 21(10): 2,691–2,707.
- Blaustein AR, Kiesecker JM. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters* 5(4): 597–608.
- Botts EA, Erasmus BFN, Alexander GJ. 2013. Small range size and narrow niche breadth predict range contractions in South African frogs. *Global Ecology and Biogeography* 22(5): 567–576.
- Chan WP, Chen IC, Colwell RK, Liu WC, Huang CY, Shen SF. 2016. Seasonal and daily climate variation have opposite effects on species elevational range size. *Science* 351(6280): 1,437–1,439.
- Chen C, Chen C, Wang Y, Liu X. 2019. Ecological correlates of extinction risk in Chinese amphibians. *Diversity and Distributions* 25(10): 1,586–1,598.
- Chen IC, Shiu HJ, Benedick S, Holloway JD, Chey VK, Barlow HS, Hill JK, Thomas CD. 2009. Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proceedings of the National Academy of Sciences of the United States of America* 106(5): 1,479–1,483.
- Cooper N, Bielby J, Thomas GH, Purvis A. 2008. Macroecology and extinction risk correlates of frogs. *Global Ecology and Biogeography* 17(2): 211–221.
- Di Marco M, Santini L. 2015. Human pressures predict species' geographic range size better than biological traits. *Global Change Biology* 21(6): 2,169–2,178.
- Fei L, Ye C, Hu S, Tian W. 2006. *Fauna Sinica. Amphibia. Volume 1. General Account of Amphibia, Gymnophiona and Urodela*. Science Press, Chinese Academy of Sciences, Beijing, China. 471 p. [In Chinese].
- Fei L, Hu S, Ye C, Huang Y. 2009a. *Fauna Sinica. Amphibia. Volume 2. Anura*. Science Press, Chinese Academy of Sciences, Beijing, China. 957 p. [In Chinese].
- Fei L, Hu S, Ye C, Huang Y. 2009b. *Fauna Sinica. Amphibia. Volume 3. Anura. Ranidae*. Science Press, Chinese Academy of Sciences, Beijing, China. 920 p. [In Chinese].
- Fei L, Ye C, Hu S, Liu C. 1976. Amphibian fauna of Sichuan. *Materials for Herpetological Research* 3:

- 1–17.
- Fei L, Ye C, Jiang J. 2012. [*Colored Atlas of Chinese Amphibians and their Distributions*]. Sichuan Publishing House of Science and Technology, Chengdu, China. 620 p. [In Chinese].
- Frishkoff LO, Gabot E, Sandler G, Marte C, Mahler DL. 2019. Elevation shapes the reassembly of Anthropocene lizard communities. *Nature Ecology and Evolution* 3(4): 638–646.
- Frost DR. 2019. Amphibian Species of the World: an Online Reference. Version 6.0. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 3 January 2019].
- Gaston KJ. 1996. Species range size distributions: patterns, mechanisms, and implications. *Trends in Ecology and Evolution* 11(5): 197–201.
- Hof C, Araujo MB, Jetz W, Rahbek C. 2011. Additive threats from pathogens, climate, and land-use change for global amphibian diversity. *Nature* 480(7378): 516–519.
- Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SH, Carpenter KE, Chanson J, Collen B, Cox NA, et al. 2010. The impact of conservation on the status of the world's vertebrates. *Science* 330(6010): 1,503–1,509.
- Hu J, Huang Y, Jiang J, Guisan A. 2019. Genetic diversity in frogs linked to past and future climate changes on the roof of the world. *Journal of Animal Ecology* 88(6): 953–963.
- Hu J, Li C, Xie F, Jiang J. 2012. Endemic amphibians and their distribution in China. *Asian Herpetological Research* 3(2): 163–171.
- Hu J, Xie F, Li C, Jiang J. 2011. Elevational patterns of species richness, range, and body size for spiny frogs. *PLoS ONE* 6(5): e19817.
- Huang Y, Wang X, Yang X, Jiang J, Hu J. 2020. Unveiling the roles of interspecific competition and local adaptation in phenotypic differentiation of parapatric frogs. *Current Zoology* 66: 383–392.
- IUCN. 2018. The IUCN Red List of Threatened Species. Version 2018-2. IUCN: Gland, Switzerland. Available: <http://www.iucnredlist.org> [Accessed: 3 January 2019].
- Jiang J, Xie F, Zang C, Cai L, Li C, Wang B, Li J, Wang J, Hu J, Wang Y, et al. 2016. Assessing the threat status of amphibians in China. *Biodiversity Science* 24(5): 588–597.
- Khatiwada JR, Zhao T, Chen Y, Wang B, Xie F, Cannatella DC, Jiang J. 2019. Amphibian community structure along elevation gradients in eastern Nepal Himalaya. *BMC Ecology* 19(1): 19.
- Korner C. 2007. The use of ‘altitude’ in ecological research. *Trends in Ecology and Evolution* 22(11): 569–574.
- Kusrini MD, Lubis MI, Endarwin W, Yazid M, Darmawan B, Ul-Hasanah AU, Sholihat N, Tajalli A, Lestari V, Utama H, et al. 2017. Elevation range shift after 40 years: the amphibians of Mount Gede Pangrango National Park revisited. *Biological Conservation* 206: 75–84.
- Li C. 1990. The natural vertical zones on the east slope of Mt. Emei in Sichuan province. *Journal of Mountain Research* 8(1): 39–44.
- Li Z, Shi L. 2007. *Plants of Mount Emei*. Beijing Science and Technology Press, Beijing, China. 492 p.
- Liu C. 1950. *Amphibians of Western China*. Chicago Natural History Museum Press, Chicago, Illinois, USA. 400 p.
- Liu S, Yang M. 2012. The biodiversity monitoring and sustainable tourism development of Mount Emei. *Sichuan Environment* 31(Suppl 1): 118–121.
- Lomolino MV. 2001. Elevation gradients of species density: historical and prospective views. *Global Ecology and Biogeography* 10(1): 3–13.
- Longino JT, Branstetter MG. 2019. The truncated bell: an enigmatic but pervasive elevational diversity pattern in Middle American ants. *Ecography* 42(2): 272–283.
- MEP (Ministry of Environment Protection), CAS (Chinese Academy of Sciences). 2015. Red List of China's Biodiversity: Amphibians. Available: <http://www.mee.gov.cn/gkml/hbb/bgg/201505/W020150526581939212392.pdf> [Accessed: 3 February 2020].
- Navas CA, Carvajalino-Fernández JM, Saboyá-Acosta LP, Rueda-Solano LA, Carvajalino-Fernández MA, Van Damme R. 2013. The body temperature of active amphibians along a tropical elevation gradient: patterns of mean and variance, and inference from environmental data. *Functional Ecology* 27(5): 1,145–1,154.
- Pearson RG, Thuiller W, Araújo MB, Martinez-Meyer E, Brotons L, McClean C, Miles L, Segurado P, Dawson TP, Lees DC. 2006. Model-based uncertainty in species range prediction. *Journal of Biogeography* 33(10): 1,704–1,711.
- Perrigo A, Hoorn C, Antonelli A. 2019. Why mountains matter for biodiversity. *Journal of Biogeography* 47(2): 315–325.
- Peters MK, Hemp A, Appelhans T, Behler C, Classen A, Detsch F, Ensslin A, Ferger SW, Frederiksen SB, Gebert F, et al. 2016. Predictors of elevational biodiversity gradients change from single taxa to the multi-taxa community level. *Nature Communications* 7: 13,736.
- Quintero I, Jetz W. 2018. Global elevational diversity and diversification of birds. *Nature* 555: 246.
- Rahbek C. 1995. The elevational gradient of species richness: a uniform pattern? *Ecography* 18(2): 200–205.
- Rahbek C. 1997. The relationship among area, elevation, and regional species richness in neotropical birds. *American Naturalist* 149(5): 875–902.

- Rahbek C. 2005. The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters* 8(2): 224–239.
- Rosenzweig ML. 1995. *Species Diversity in Space and Time*. Cambridge University Press, London, United Kingdom. 460 p.
- Tang CQ. 2006. Forest vegetation as related to climate and soil conditions at varying altitudes on a humid subtropical mountain, Mount Emei, Sichuan, China. *Ecological Research* 21(2): 174–180.
- Tang CQ, Ohsawa M. 1997. Zonal transition of evergreen, deciduous, and coniferous forests along the altitudinal gradient on a humid subtropical mountain, Mt. Emei, Sichuan, China. *Plant Ecology* 133(1): 63–78.
- Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105(Suppl 1): 11,466–11,473.
- Wang X, Huang Y, Zhong M, Yang S, Yang X, Jiang J, Hu J. 2019. Environmental stress shapes life-history variation in the Swelled-vented Frog (*Feirana quadranus*). *Evolutionary Ecology* 33(3): 435–448.
- Wells KD. 2007. *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago, Illinois, USA. 1,400 p.
- Xie F, Jiang J, Zheng Z, Liu J. 2007. An analysis of the species diversity and distribution patterns of amphibians in Gongga Shan Mountain area. *Acta Herpetologica Sinica* 11: 25–32.
- Zhao B, Chen R. 1980. *Mount Emei*. Sichuan People's Press, Chendgu, China. 68 p.
- Zhao T, Wang B, Shu G, Li C, Jiang J. 2018. Amphibian species contribute similarly to taxonomic, but not functional and phylogenetic diversity: inferences from amphibian biodiversity on Emei Mountain. *Asian Herpetological Research* 9(2): 110–118.



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Amphibians on Mount Emei, China

Appendix. Locations of sampling sites and the numbers of line transects (N) at each site along the elevational gradient on Mount Emei, China.

	Sampling site	Longitude (°E)	Latitude (°N)	Elevation (m)	N
Line transects	Huangwan Village	103.43	29.58	500	2
	Baoguo Temple	103.44	29.57	530	2
	Lianghekou	103.41	29.59	650	1
	Qingyin Pavilion	103.39	29.57	730	1
	Shenshui Pavilion	103.41	29.56	800	2
	Baiguo Village	103.34	29.43	860	1
	Chadi Village	103.36	29.59	914	1
	Weigan Village	103.31	29.60	1,100	1
	Longdong Village	103.28	29.58	1,250	2
	Qiliping	103.25	29.57	1,280	1
	Linggongli	103.29	29.58	1,340	2
	Kuhaoping	103.27	29.45	1,470	2
	Changshou Bridge	103.35	29.56	1,540	1
	Jinchuan Village	103.24	29.44	1,560	2
	Longqiaogou	103.35	29.55	1,900	1
	Jingding	103.33	29.52	3,050	1
Sampling points	Shouxing Bridge	103.37	29.55	1,280	
	Shuangshuijing	103.32	29.55	2,230	
	Leidongping	103.33	29.55	2,433	



New record of *Adelphicos daryi* (Serpentes: Dipsadidae) after 19 years, and additional record of *Ptychohyla euthysanota* (Anura: Hylidae) in Guatemala

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Abstract.—New records of the Endangered *Adelphicos daryi* (Serpentes: Dipsadidae) and Near Threatened *Ptychohyla euthysanota* (Anura: Hylidae) are reported for the Department of Guatemala, Guatemala City. Brief comments on local conservation concerns for these two species are presented.

Keywords. Amphibia; Central America; Endangered; new records; Reptilia

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Snakes of the genus *Adelphicos* are small (39–57 cm) and secretive leaf litter inhabitants of rainforest, cloud forest, and pine-oak and conifer forest habitats, and they range from northern Mexico (Tamaulipas) to western Honduras (Campbell and Ford 1982; Kholer 2008). Six species of *Adelphicos* are registered for Guatemala. Within this genus, *Adelphicos daryi* was described by Campbell and Ford in 1982, based on 11 specimens reported from San Jorge Muxbal, Department of Guatemala, Guatemala (Campbell and Ford 1982).

The IUCN Red List classifies *Adelphicos daryi* as Endangered due to its very restricted distribution in the central Guatemalan highlands. The species is terrestrial, fossorial, and mainly nocturnal (Acevedo et al. 2014). Prior to the report made in this paper, there were five known localities for this species based on many records all within the Department of Guatemala: San Jorge Muxbal (type locality), Villa Canales and Las Joyas, Santa Catarina, Pinula; Km 14.5 carretera a El Salvador, Puerta Parada; Vista Hermosa III, Universidad Rafael Landivar, Zona 15, and San Rafael, carretera a El Salvador (Table 1) [J. Campbell, pers. comm. 2019].

The genus *Ptychohyla* includes frogs that inhabit broad-leaf and mixed pine forest, with six species recognized in Central America (Faivovich et al. 2018). There are six species vouchered for Guatemala, including *Ptychohyla euthysanota* (Kellogg, 1928). The IUCN Red List classifies *Ptychohyla euthysanota* as Near

Threatened because its distribution is not much greater than 20,000 km² (Santos-Barrera et al. 2010).

Observations

During a nocturnal photography hike in the Río Las Monjas on 11 July 2017 at 2100 h, the junior author observed a snake alongside a large rock. It was initially believed to be *Stenorrhina freminvillei*. However, following more careful examination of the living specimen, it was photographed (Fig. 1), and the images were forwarded to Jonathan Campbell of the University of Texas at Arlington for identification purposes. Dr. Campbell confirmed that the specimen was *Adelphicos daryi*. This record represents a new location for the Department of Guatemala: Guatemala City, zona 16, Río Las Monjas; 14.6075°N -90.463055°W (WGS84), 1,530 m asl (Fig. 2). Although this site is not a great distance from the previous reported localities (Table 1), it has been 19 years since the last confirmed report of this species from Km 14.5 Carretera a El Salvador, Puerta Parada.

The same night, the senior author observed a male of *Ptychohyla euthysanota* calling from a small shrub beside the Río Las Monjas at 2244 h (Fig. 3). This observation and photographic confirmation are separated by 21.25 km airline from the nearest prior record of this species in the Lago de Amatitlán (based on IUCN Records) [Fig. 4].

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Conservation Concerns

Unfortunately, these new records for *Adelphicos daryi* and *Ptychohyla euthysanota* both occur in an unprotected area in Guatemala City. Access to the new locality is restricted in some parts for urban settlements, while for other parts the access is easy, and some local people still extract firewood from this area. The major threats that *Ptychohyla euthysanota* currently faces are alteration or loss of original habitats due to agricultural activity, logging (Santos-Barrera et al. 2010), and the pollution of water bodies where they breed (Fig. 5). Another threatened amphibian has previously been recorded at this locality, *Plectrohyla guatemalensis*, which is classified by the IUCN as Critically Endangered. Conservation efforts need to be improved in this area, in order to safeguard this endemic snake and regionally endemic frog. These efforts should work with the communities, to promote the creation of a comprehensive national water law, and reduce deforestation (Acevedo et al. 2014).

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Literature Cited

- Acevedo M, Ariano-Sánchez D, Johnson J. 2014. *Adelphicos daryi*. *The IUCN Red List of Threatened Species* 2014: e.T521A3014000.
- Campbell JA, Ford LS. 1982. Phylogenetic relationships of the colubrid snakes of the genus *Adelphicos* in the highlands of Middle America. *Occasional Papers of the Museum of Natural History University of Kansas* 100: 1–22.
- Faivovich J, Pereyra MO, Luna MC, Hertz A, Blotto BL, Vásquez-Almazán CR, Köhler G. 2018. On the monophyly and relationships of several genera of Hylini (Anura: Hylidae: Hylinae), with comments on recent taxonomic changes in hylids. *South American Journal of Herpetology* 13(1): 1–32.
- Köhler G. 2008. *Reptiles of Central America*. 2nd Edition. Herpeton-Verlag Elke Köhler, Offenbach, Germany. 400 p.
- QGIS Development Team. 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available: <http://qgis.osgeo.org> [Accessed: 3 June 2019].
- Santos-Barrera B, Acevedo M, Muñoz Alonso A. 2010. *Ptychohyla euthysanota*. *The IUCN Red List of Threatened Species* 2010: e.T55910A11388786.

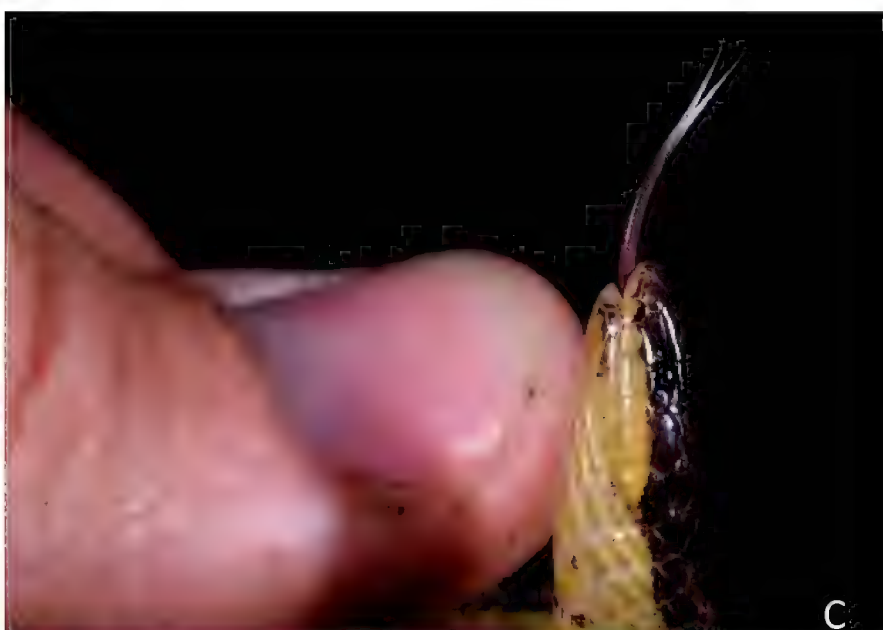


Fig. 1. Individual of *Adelphicos daryi* in life. (A) Ventral view and (B) dorsal pattern of the specimen; (C) comparison of the size of the head with author's thumb; (D) lateral view of the head. Photos by Fred Muller.

Distribution of *Adelphicos daryi* in Guatemala.

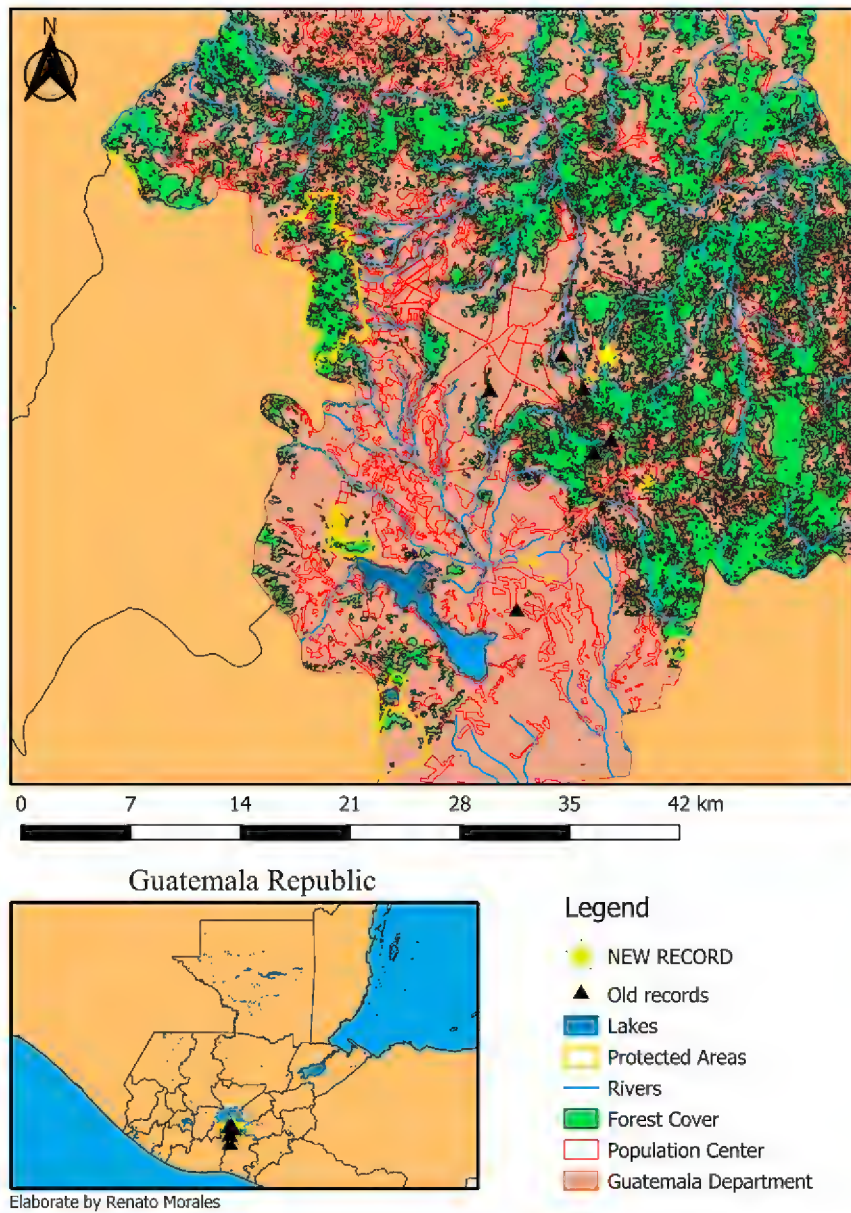


Fig. 2. Map of the distribution of *Adelphicos daryi* in Guatemala. Yellow star: new record; black circles: previous records; brown area: Guatemala Department. The map was developed with QGIS software (QGIS Development Team 2020).

Distribution of *Ptychohyla euthysanota* in Guatemala.

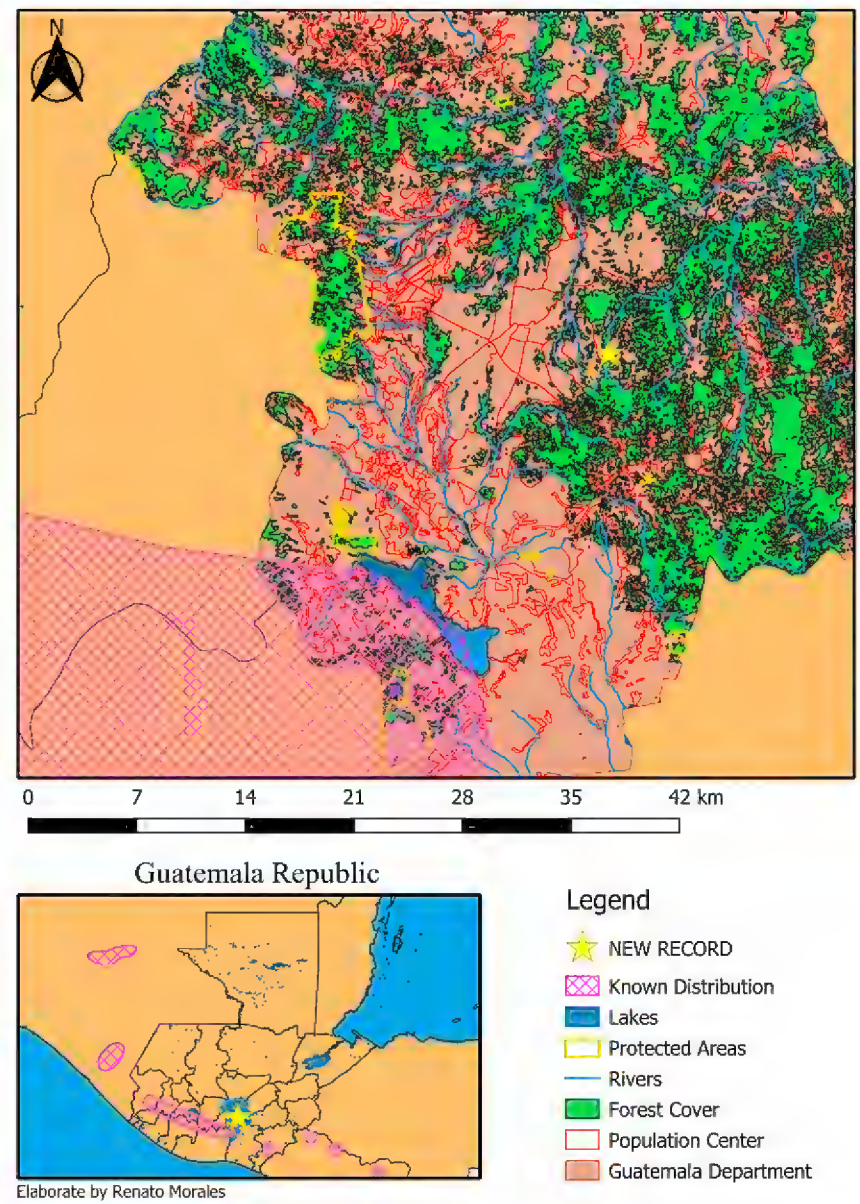


Fig. 4. Map of the distribution of *Ptychohyla euthysanota* in Guatemala. Yellow star: new record; purple polygons: previous records; brown area: Guatemala Department. The map was developed with QGIS software (QGIS Development Team 2020).

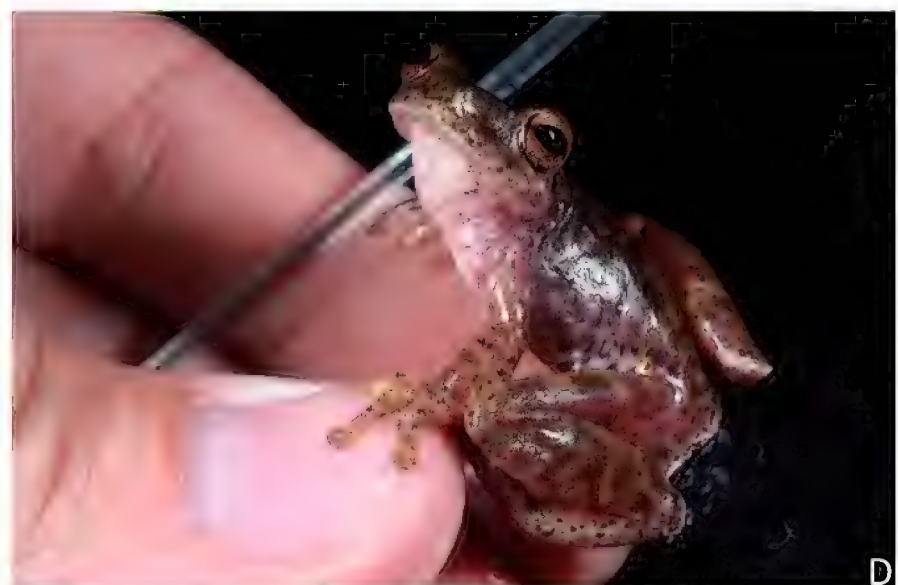
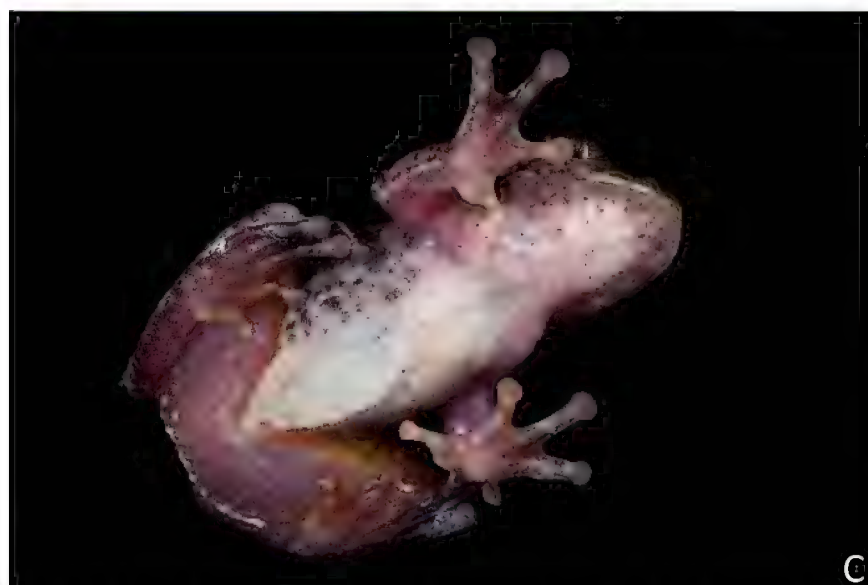


Fig. 3. Male of *Ptychohyla euthysanota* in life. (A) Lateral, (B) frontal, (C) ventral, and (D) lateral views of the specimen. Photos by Renato Morales (A–B) and Fred Muller (C–D).

New records of *Adelphicos daryi* and *Ptychohyla euthysanota*

Table 1. Historical and new records of *Adelphicos daryi* for Guatemala. UTA: University of Texas Arlington; UVGR: Univerisdad del Valle de Guatemala.

	Voucher	Locality	Latitude	Longitude	Capture date
Previous records	UVGR 2575	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	1980
	UVGR 1131	Km 14.5 carretera a El Salvador, Puerta Parada, frente a Iglesia Sn. Francisco	14.5575	-90.4611	1984
	UVGR 1088	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	1987
	UVGR 1376	Km 14.5 carretera a El Salvador, Puerta Parada, frente a Iglesia Sn. Francisco	14.5575	-90.4611	4 May 1975
	UVGR 1389	Km 14.5 carretera a El Salvador, Puerta Parada, frente a Iglesia Sn. Francisco	14.5575	-90.4611	4May 1975
	UVGR 246	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	13 Dec 1979
	UVGR 3	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	6 Nov 1980
	UVGR 226	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	2 Jul 1982
	UVGR 380	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	30 May 1984
	UVGR 1194	Km 14.5 carretera a El Salvador, frente a Iglesia San Francisco	14.5575	-90.4611	13 Aug 1984
	UVGR 1197	Km 14.5 carretera a El Salvador, Puerta Parada, frente a Iglesia Sn. Francisco	14.5575	-90.4611	27 Sep 1984
	UVGR 535	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	10 Apr 1985
	UVGR 1508	Km 14.5 carretera a El Salvador, Puerta Parada, frente a Iglesia Sn. Francisco	14.5575	-90.4611	1 Aug 1990
	UVGR 1844	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	24 Apr 1991
	UVGR 1845	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	24 Jun 1991
	UTA 32950	Villa Canales, Villa Tapacon, Quebrada Norte			12 May 1992
	UVGR 1991	zona 15, Vista Hermosa III, Universidad Rafael Landivar	14.6065	-90.4901	27 Aug 1992
	UVGR 1840	zona 10, Colonia El Prado	14.5864	-90.5328	21 Sep 1992
	UVGR 2023	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	8 Nov 1992
	UVGR 2032	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	15 Jul 1994
	UVGR 2033	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	10 Sep 1994
	UVGR 2872	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	10 Sep 1994
	UVGR 3084	San Rafael, carretera a El Salvador	14.3333	-90.5167	28 Oct 1994
	UTA 39216	Santa Catarina Pinula, San Miguel Buena Vista (Las Joyas)			11 Nov 1994
	UVGR 2059	Km 14.5 carretera a El Salvador	14.5575	-90.4611	2 Jul 1995
	UVGR 2142	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	8 Dec 1995
	UVGR 2356	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	15 May 1997
	UVGR 2355	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	8 Oct 1997
	UVGR 2809	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	5 Mar 1998
	UVGR 2572	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	No data
	UVGR 2741	Km 14.5 carretera a El Salvador, Puerta Parada	14.5575	-90.4611	No data
New Record	No voucher	Río Las Monjas, Zona 16, Guatemala City	14.6075	-90.463055	11 Jul 2017

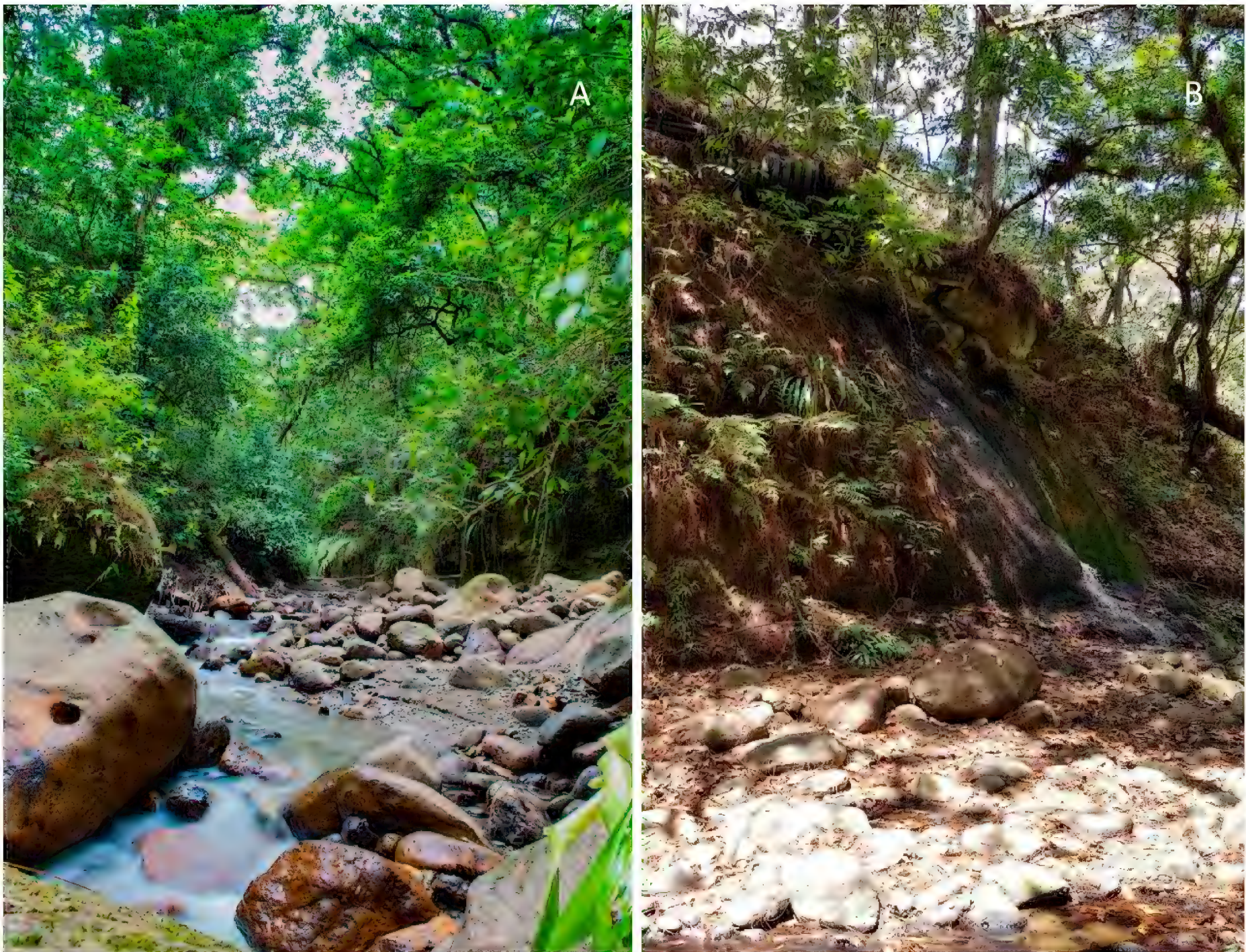


Fig. 5. Río Las Monjas, Zona 16, Guatemala City. **(A)** Habitat for *Adelphicos daryi* and *Ptychohyla euthysanota*; **(B)** Pollution of the waterbody by drainage.



Renato Morales is a Guatemalan biologist and herpetologist, and amateur wildlife photographer. He has been learning about and working on amphibians and reptiles in Guatemala since 2013. Renato served as a teaching assistant in evolution courses, and director of geology and paleontology courses, at the Universidad de San Carlos de Guatemala; and he belongs to the group of taxonomists of the National Council for Protected Areas (CONAP). In 2019, he was invited to the IUCN Red List Workshop as part of the Guatemalan amphibian specialist group, and he is currently working on the genetics of lizards and the conservation of salamanders and frogs.



Fred Muller has had a lifelong interest in natural history and began his career as a nature photographer, specializing in botany, with a post at the Lyon Botanical Garden in France. He worked there as staff photographer from 2002 to mid-2007, when he moved to Guatemala. Since then, Fred has worked as an ecotourism guide and nature photographer specializing in Mesoamerican biodiversity. His current topics of interest include the region's most endangered flora and herpetofauna. Fred has accumulated a substantial collection of unique portraits of plants and animals, and his photography has been showcased in many scientific publications as well as in public showings. Recently, his photographs have appeared on websites such as *Exotica Esoterica* (<http://www.exoticaesoterica.com>) where he is an author and photographer. Many examples of his work can be seen on his Flickr page (<http://www.flickr.com/photos/fredmullerpix>) as well as at *Aroid Pictures* (<http://www.aroidpictures.fr>).



The need for transboundary faunistics and conservation: first record of the Natterjack Toad (*Epidalea calamita*) in Czech Silesia, northeastern Czech Republic

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Abstract.—The Natterjack Toad (*Epidalea calamita*) has been severely declining in the northern and eastern parts of its range in past decades. An immense population decline has been recorded in the Czech Republic, the southeastern edge of the species range. Contrary to the majority of published distribution range maps of the Natterjack Toad, it is present only in the western part of the Czech Republic (Bohemia), scattered among mostly isolated populations. A new, relatively distant population was recently discovered in the northeastern part of the country, in Czech Silesia. The genetic analysis presented here demonstrates that the new population belongs to the evolutionary lineage that is widely distributed in the northeastern part of the species range. Thus, this population is not a possible exotic introduction, but probably represents a natural extension of Natterjack Toad populations from Poland to the south. We urge conservation actions to be taken immediately to support this unique population, which is presently inhabiting a dump site. We further emphasize the necessity of considering distribution records on both sides of state borderlines when faunistic research is conducted in borderlands.

Keywords. Amphibians, anthropogenic habitat, Bufonidae, Central Europe, distribution, edge populations, geographic range limit, phylogeography, range extension

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Introduction

The Natterjack Toad, *Epidalea calamita* (Laurenti, 1768), Bufonidae, is native to southwestern through northern Europe, and parts of eastern Europe (from Portugal to southern Sweden, Estonia, Belarus, and northwestern Ukraine), where it typically occupies sunny open sandy areas and heathlands (Sillero et al. 2014; Sinsch 2009). The species also tolerates saline habitats to some extent, including sand dunes on sea shores, and a substantial part of its range corresponds to the oceanic climate. However, within the continental climate zone it is more commonly found in anthropogenic habitats, such as agricultural areas like vegetable crop fields (Zavadil et al. 2011) and quarries (Arnold 2002; Speybroeck et al. 2016), due to the lack of natural habitats, such as floodplains. In the northern and eastern parts of the range, including Central Europe, the abundance of the Natterjack Toads has drastically declined in past decades (Dufresnes 2019).

In the Czech Republic (= Czechia), the Natterjack Toad is one of three bufonids and the rarest and most threatened anuran species, being registered as Critically Endangered (Jeřábková et al. 2017, 2019; Šandera et al. 2017). It is present only in Bohemia, the western part of the Czech Republic (Jeřábková and Zavadil 2020; Moravec 2019; Sinsch 2009; Vitáček and Zavadil 1994; Zavadil 1994, 1996), contrary to the majority of published species range maps in books and conservation/research resources (e.g., Beja et al. 2009; Sillero et al. 2014), which incorrectly show the range across the whole Czech Republic. The distribution in Czechia represents the southeastern edge of the species range (Beja et al. 2009; Sillero et al. 2014; Sinsch 2009). The distribution in Czechia is very scattered, with most of the populations isolated from each other (Fig. 1; AOPK ČR 2019; Jeřábková and Zavadil 2020; Maštera et al. 2015; Šandera et al. 2017), and on the edge of survival (Jeřábková et al. 2017, 2019; Šandera et al. 2017). A relatively continuous distribution in Czechia is present only in the Cheb and Sokolov Basins (Jeřábková

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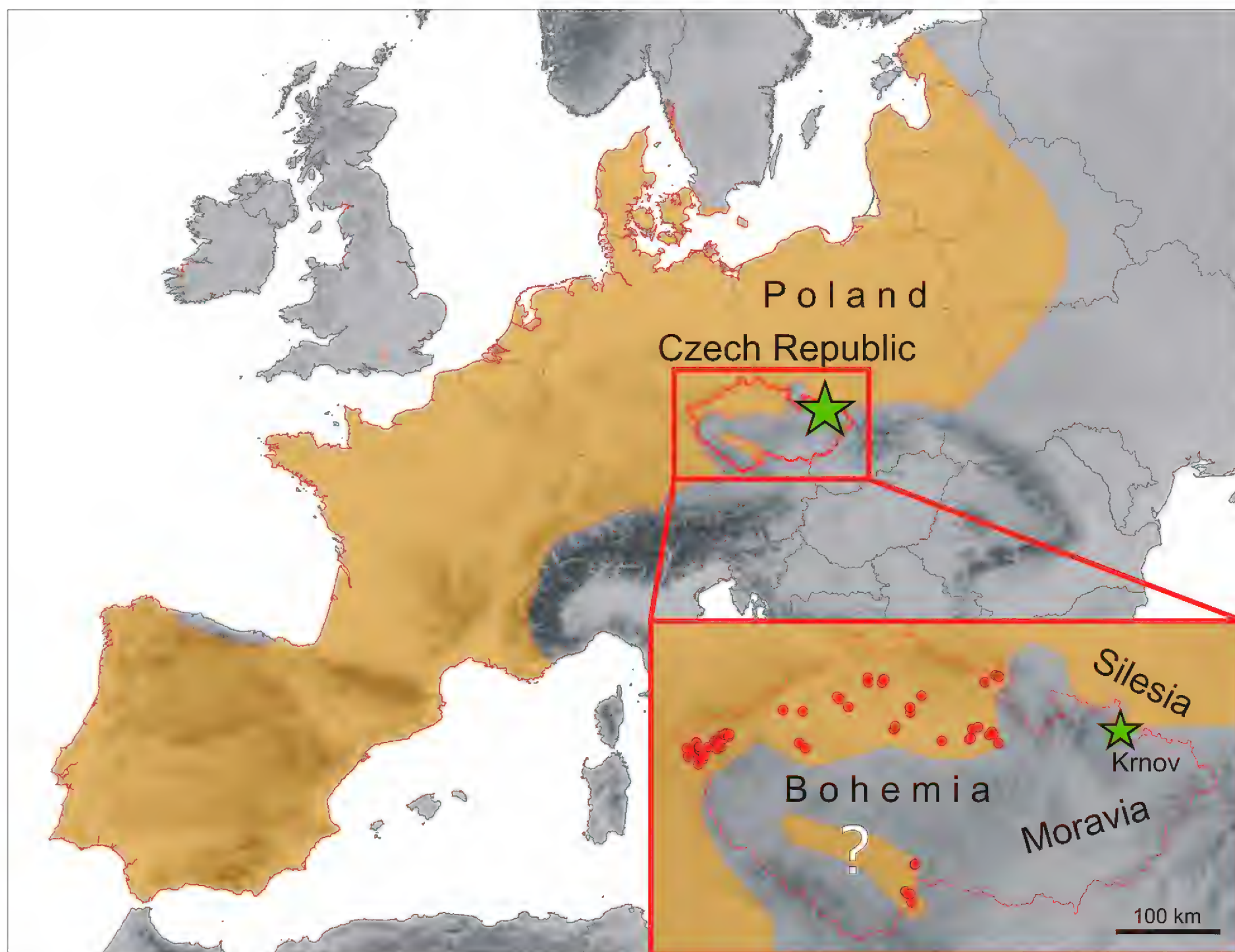


Fig. 1. Distribution range of the Natterjack Toad (*Epidalea calamita*) according to the *IUCN Red List of Threatened Species* (Beja et al. 2009), updated for the Czech Republic (inset). Red dots show all recent confirmed records (1997–2016; Šandera et al. 2017), demonstrating the very scattered distribution in the Czech Republic. The question mark denotes the region where the Natterjack Toad had occurred before 1990, but has since disappeared (AOPK ČR 2019; Jeřábková and Zavadil 2020). The green star shows the newly discovered population in Krnov, Czech Silesia.

and Zavadil 2020; Zavadil et al. 2011). The Natterjack Toad has never been reported reliably from Moravia or Czech Silesia, the eastern and northeastern parts of the Czech Republic (Šandera et al. 2017; Zavadil 1994, 1996). However, the recently published distribution map of the Natterjack Toad in Poland (Profus and Sura 2018) documents its occurrence in Polish Silesia (southern Poland), which indicates the possible presence of the Natterjack Toad on the other side of the border in Czech Silesia.

The presence of *E. calamita* in Czech Silesia was recently confirmed in Krnov, in the borderlands with Poland, highlighting the importance of transboundary faunistic studies. The site is less than 10 km from the nearest Polish locality. In this study, the genetic identity of the newly discovered population was tested to determine whether it was introduced from an exotic site. Specifically, the test can determine if the population originated in southwestern Europe, which hosts evolutionary lineages that differ from the lineages located in Central Europe (Rowe et al. 2006; Zeisset and Beebee 2014). The new

locality is described, the historic and present threats are discussed, and a baseline is proposed for the conservation management of this newly discovered population.

Materials and Methods

Study Area

The newly discovered locality, Krnov-Cvilín (50.0703°N, 17.7192°E, 390 m asl; Figs. 1–2), lies in the borderlands of the Czech Republic and Poland, in the foothills of the Jeseníky Mountains (northeastern part of the Bohemian Massif), and at the edge of the Silesian Lowlands. The area is a mosaic of forested and agricultural landscapes, which changes into a predominately agricultural landscape in the Silesian Lowlands.

Genetic Methods

Genomic DNA was extracted from toe clips of five Natterjack Toads using a spin column-based extraction



Fig. 2. Newly discovered locality of the Natterjack Toad (*Epidalea calamita*) in Krnov, Czech Silesia, Czech Republic. The distribution site is located within the Cvilín demolition waste dump, which used to be a sand quarry. **(A)** The site is now completely filled-up by demolition waste, an unfavorable condition for several species of amphibians present at the site. **(B)** Puddles are formed in small depressions after the movement of heavy-weight vehicles. However, the formation of such small puddles will probably stop in the near future as the waste dump is now closed. This is another unfavorable condition for this population, together with the surrounding grounds being overgrown by dense vegetation.

kit and following the manufacturer's manual. One sample was from a subadult specimen from the newly discovered population (Fig. 3) in the northeast of the Czech Republic (Krnov), while four samples for comparison were from the western Czech Republic (Odrava-Obilná, sand quarry; 50.1030°N, 12.4732°E, 425 m asl). PCR amplification and DNA sequencing targeted a fragment of 16S rRNA using the primers and protocol reported by Gvoždík et al. (2010). The nucleotide sequences obtained were supplemented by available conspecific 16S rRNA data from GenBank. The GenBank data were derived from 24 individuals from throughout the species distribution range, including one additional sample from the western Czech Republic (GenBank KF665137; Lomnice, Erika sand quarry; 50.2117°N, 12.6064°E, 480 m asl) and two outgroup taxa (*Bufo bufo* and *Bufotes viridis*, both from Czechia). GenBank numbers and countries of origin are given in Fig. 4, and the new sequences were deposited in GenBank (MT396931–MT396935). Alignment of the nucleotide sequences was prepared using the Mafft algorithm (Kato and Standley 2013), with default settings as incorporated in Geneious R8.1 (Biomatters, Auckland, New Zealand), and included 548 aligned sites. The maximum-likelihood phylogenetic tree was constructed by the RAxML algorithm (Stamatakis 2014) using the general time-reversible model of substitution evolution with rate heterogeneity, and 100 bootstrap pseudoreplicates to assess the branching supports.

Results and Discussion

Discovery of the New Population and Site Characteristics

In July 2019, an amateur naturalist found several specimens of the Natterjack Toad in a demolition waste dump in Krnov-Cvilín. The toads were originally erroneously identified as the Green Toad (*Bufotes*

viridis), and later re-identified according to photographs by the first author of this contribution. The finding was popularized in internet media as a finding of “the rarest Czech anuran in a dump” (Kuba 2019). On 8 August 2019 at 2110–2220 h, with an air temperature of 18–20 °C, six specimens (four adults, one older subadult, and one younger subadult) of *E. calamita* were found at the dump site in Krnov, Cvilín Quarter, which represented the first confirmed record of the Natterjack Toad in Czech Silesia (Fig. 3). Subsequent surveys in August and September 2019 brought more findings of around ten individuals of different ages, including both adults and subadults. Together with the finding of the younger subadult (Fig. 3D), the various ages of the individuals suggest that the population was then – or until recently had been – reproducing.

The distribution site in Krnov-Cvilín (Fig. 1) is approximately 2.3 km from the border with Poland, which is formed by the Opava River. Climatically, the site is in a warmer region of the Czech Republic (Quitt 1971), with an average temperature in January (coldest month) of -2.6 °C and in July (warmest month) of 17.0 °C (<https://en.climate-data.org/>). Geomorphologically, the site is within the Zlatohorská Highlands, a part of the Jeseníky Mountains. According to the Kartierung der Flora Mitteleuropas (KFME) mapping grid, a widely used floristic and faunistic mapping system in the Czech Republic, the Cvilín dump lies within grid cell #5972 (Pruner and Mika 1996).

Genetic Identity

The specimen from the Krnov population has the same haplotype as the specimens from Western Bohemia (Czechia), as well as specimens from northern France, the Netherlands, and Denmark (Fig. 4). Closely related haplotypes originated from Sweden. In the Western Bohemian site (Odrava-Obilná), one additional haplotype

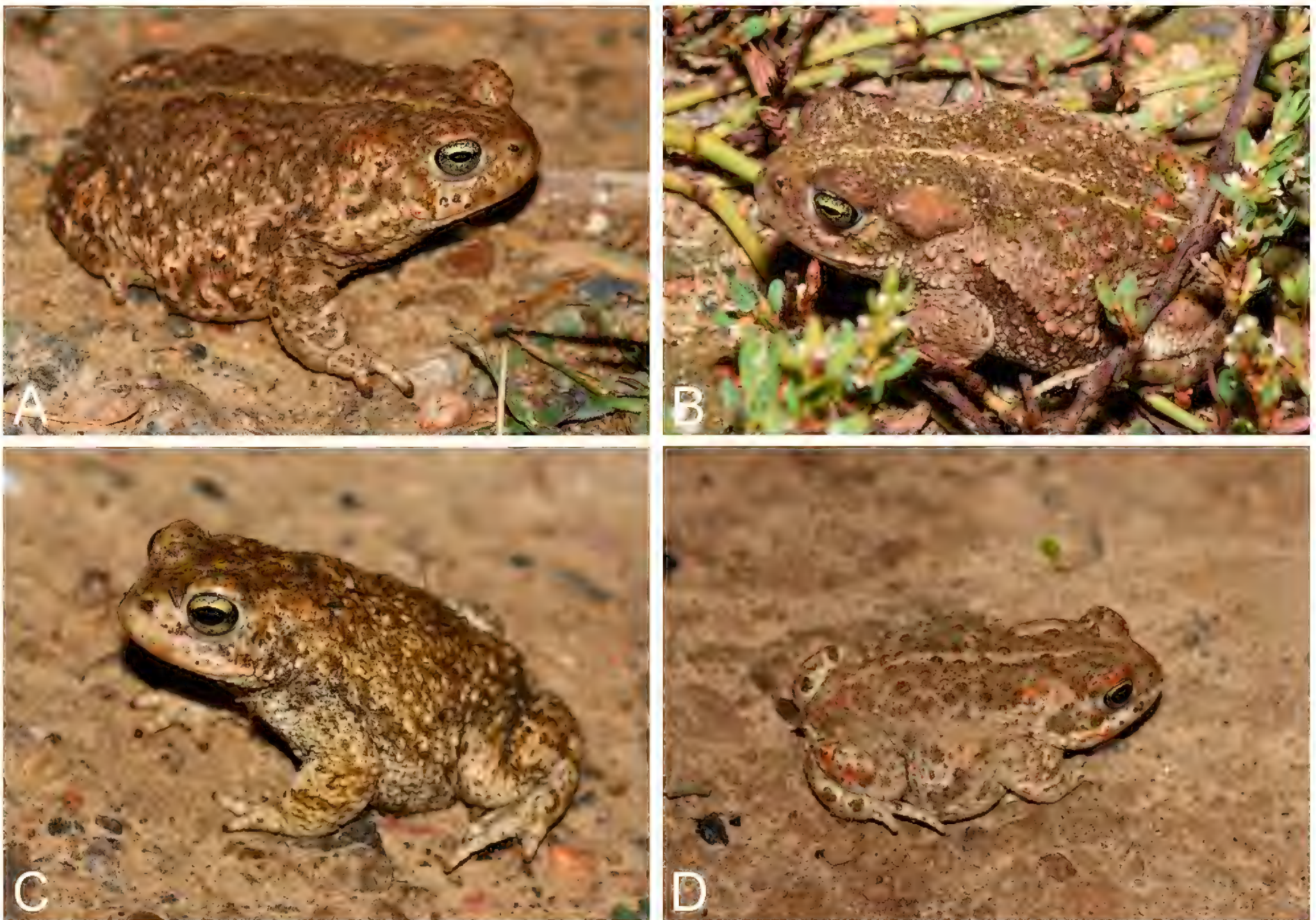


Fig. 3. Individuals of the Natterjack Toad (*Epidalea calamita*) from the newly discovered population in Krnov, Czech Silesia, Czech Republic, all found active at night in August 2019. **(A)** Adult female (SVL 74 mm), **(B)** genetically tested subadult specimen, **(C)** adult male with an indistinct dorsal stripe, and **(D)** the smallest subadult (SVL 34 mm) that was found.

was found. All of these haplotypes form a well-supported clade, which corresponds to Clade A according to Rowe et al. (2006). A comparison of the results of this study with those of Rowe et al. (2006) and Zeisset and Beebee (2014), indicates that Clade A is distributed from northern Spain and western and northern France to Britain, Ireland, the Netherlands, Denmark, Sweden, Estonia, and Central Europe, including Germany, Poland, and the Czech Republic. A higher genetic diversity in the Natterjack Toad is found only in the Iberian Peninsula, southern France, and partially in Switzerland and southwestern Germany (Fig. 4; Rowe et al. 2006). The remainder of the range, including the Czech Republic, is occupied by a genetically relatively uniform evolutionary lineage, which probably colonized this region during the post-glacial expansion (Rowe et al. 2006; Zeisset and Beebee 2014).

For effective conservation management, it is important to point out that the specimen from Krnov is a member of this widespread Central and Northern European lineage (Clade A *sensu* Rowe et al. 2006), which is also native in Czechia. This means that the Krnov population is not a distant, exotic introduction, specifically from southwestern Europe (e.g., southern Iberia or southern France). The most probable hypothesis for this population's occurrence in Krnov is an autochthonous

origin with biogeographic and evolutionary connections to populations in the Silesian Lowlands in Poland. However, the application of a population-genetic approach is needed to more precisely elucidate the eco-evolutionary relationships of the Krnov population to the nearest neighboring populations both to the north in Poland and to the west in Bohemia. This will be a crucial step for properly defining conservation units, which is an essential step for the conservation management of the Natterjack Toad in the Czech Republic.

Recent History of the Locality

The site is presently a demolition waste dump that is in the process of being closed because its capacity is now full (Fig. 2). According to the town chronicle (F. Kuba, September 2019, pers. comm.) and long-time residents' memories, the site was an active sand quarry before World War II. A small pond was present when the quarry was abandoned. At the end of the 1960s, ecological degradation began when liquid toxic waste was first deposited in the abandoned quarry, and this continued until 1983. Since 1985, the site has been "recultivated" in several serial attempts which were interrupted by the continuing usage of the site as a dump. The "recultivation" landfilling was done mainly using demolition waste and

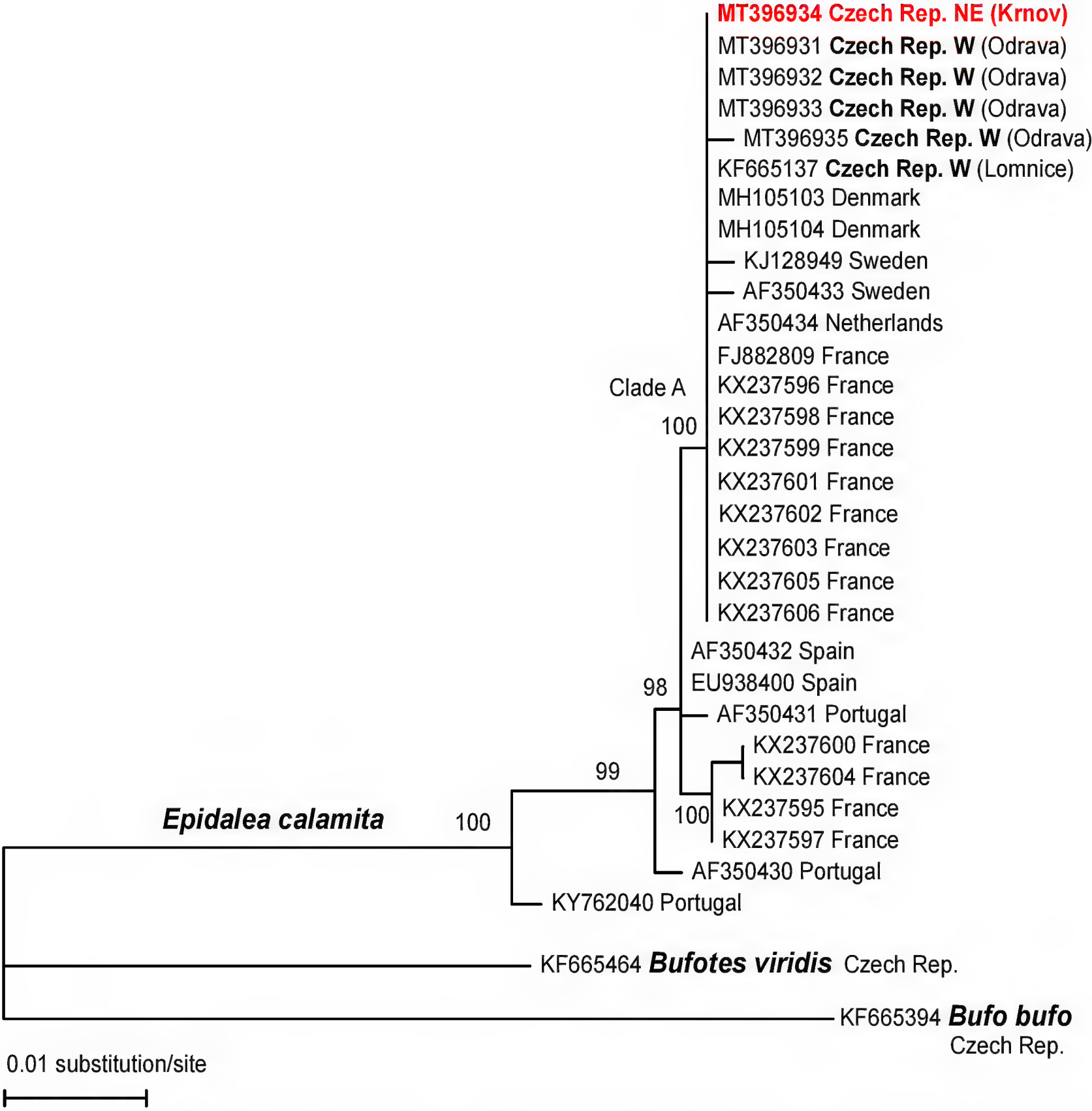


Fig. 4. Maximum-likelihood phylogenetic tree showing the position of an individual from Krnov (in red) within the Clade A (*sensu* Rowe et al. 2006), indicating the non-exotic origin of the Krnov population. All other Czech samples (from Western Bohemia) are in bold. Codes correspond to GenBank numbers, and numbers at nodes are bootstrap support values

soil. The last remains of the pond had been visible until 1997, when it was completely covered by the landfilling. However, puddles (as potential breeding sites) were probably formed after rains. The last, final phase of landfilling began in 2007 and had been ongoing until October 2019, when the dump was formally closed due to its full capacity.

Present Situation and Threats

The present size of the Cvilín dump is approximately 100 × 250 m, and the main anthropogenic disturbance is the frequent usage of the site by heavy-weight vehicles, similar to the open-pit mines of Western Bohemia (Sokolov Basin) where the Natterjack Toad is still relatively common (Zavadil et al. 2011). The site is filled with rubble, other demolition waste, and soil, with the depth of the waste layer of about 5–10 m. Some

pioneer vegetation is growing on the soil surface, and the Natterjack Toads are commonly found active near the Knotgrass (*Polygonum arenastrum*) growth, where they hide when disturbed. The ridden, smooth soil surface allows for the formation of small puddles after heavy rains. Although highly probable, it is not clear whether they are sufficient for the reproduction of the Natterjack Toads. Within the site, there is presently a single small artificial pond made from a black plastic waterproof sheet (approximately 5 × 3.5 m, maximum depth ~0.3 m). This pond was built by the local municipality to provide a breeding habitat for the local amphibian population (without the previous knowledge of the Natterjack Toad’s presence). However, this single small artificial pond is not large enough to provide a reproduction site for all the local amphibians. Moreover, it is unclear which species can utilize this pond for reproduction. Tadpoles of *Pelobates fuscus* and small postmetamorphic

juveniles of *Pelophylax kl. esculentus* were found in the pond in August 2019, suggesting that these two species are able to breed in this sole artificial pond of limited size and volume. In addition, *Bombina variegata*, *Bufo bufo*, and *Bufo viridis* were recorded within the Cvilín dump site, but it is not clear whether they successfully breed within the dump. [However, see the **Note** at the end of this article for updated information.] Krnov-Cvilín is thus one of the few sites in Czechia, where the three bufonids occur syntopically. The other amphibians known from the whole Krnov region are: *Hyla arborea*, *Pelophylax ridibundus*, *Rana arvalis*, *R. dalmatina*, *R. temporaria*, *Salamandra salamandra*, *Ichthyosaura alpestris*, *Lissotriton montandoni*, *L. vulgaris*, and *Triturus cristatus* (AOPK ČR 2019; Jeřábková and Zavadil 2020; Moravec 1994; Šiffner 2011).

Recommendations for Conservation Management

Conservation actions for the Natterjack Toad in the Cvilín dump are in preparation. Briefly, four initial recommendations are: (1) conserve the present pedologic conditions in the majority of the soil surface, but remove the demolition waste to uncover the original substratum in a part of the area; (2) retain water by building several shallow water reservoirs of different sizes and depths for reproduction, ideally on the original substratum (with up to 50 cm depth and slightly gradually sloping banks); (3) block secondary succession to avoid the overgrowth of dense and/or high vegetation; and (4) eliminate potential revegetation actions.

Importance of Transboundary Faunistics and Conservation

The newly discovered locality extends the known distribution area of *E. calamita* in the Czech Republic by 110 km (by air) east of the nearest registered location, which is a sand quarry near Plchovice-Smetana in Eastern Bohemia (50.0526°N, 16.1869°E; grid cell #5963; AOPK ČR 2019). Thus, the Natterjack Toad is newly listed as a species present in Czech Silesia. The newly discovered locality in Krnov is the easternmost known distribution site of the species in the Czech Republic, and forms the southeastern margin of the distribution range of the species in Central Europe. These individuals represent a unique Natterjack Toad population within the Czech Republic, geographically separated from the Bohemian populations, and therefore of particular conservation importance. However, it is possible that more populations are present in Czech Silesia or Northern Moravia which have yet to be discovered [see **Note** at the end of this article]. The Krnov population has a biogeographic affinity to the Polish Upper Silesian populations (see maps in Profus and Sura 2018, and the online atlas at <http://www.iop.krakow.pl/PlazyGady>). The nearest presently known distribution site is approximately 8

km to the northeast (behind the Opava River), in a sand quarry near Zubrzyce, Poland (50.1274°N, 17.7787°E, 290 m asl; grid cell #5872), a locality discovered only recently in 2018 (M. Pabijan, September 2019, pers. comm.). However, other Upper Silesian localities in Poland, in the borderlands with the Czech Republic, have been reported (Profus and Sura 2018; Świerad 1998). These earlier findings could serve as an indication of the possible presence of the Natterjack Toad in Czech Silesia, which would allow the discovery of the Krnov-Cvilín locality in a more favorable condition, before the original sand quarry was completely destroyed. Considering the distance between the Krnov population and the nearest previously known locality, we can assume that the two populations (and possibly other nearby populations) are probably genetically connected within a metapopulation network (Sinsch 2017).

This case highlights the importance and need for transboundary faunistics and conservation in general. All too often, local or national faunistics and conservation actions are conducted without proper knowledge of the situation that is right on the other side of the country borderline. Considering that species distributions in neighboring countries can bring new important discoveries (e.g., Najbar et al. 2011; Strachinis et al. 2019; Viček et al. 2010), we urge all those involved to consider the situations on both sides of borderlines when faunistic research and conservation actions are conducted in borderland areas.

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Literature Cited

Arnold EN. 2002. *Reptiles and Amphibians of Europe*. Second Edition. Princeton University Press, Princeton, New Jersey, USA and Oxford, United

- Kingdom. 288 p.
- AOPK ČR [Nature Conservation Agency of the Czech Republic]. 2019. Nálezová databáze ochrany přírody [On-line database of distribution records for nature conservation]. Available: <https://portal.nature.cz/nd> [Accessed: 1 October 2019].
- Beja P, Kuzmin S, Beebee T, Denoel M, Schmidt B, Tarkhnishvili D, Ananjeva NB, Orlov NL, Nyström P, Ogrodowczyk A, et al. 2009. *Epidalea calamita* (erratum version published in 2016). *The IUCN Red List of Threatened Species* 2009: e.T54598A86640094.
- Dufresnes C. 2019. *Amphibians of Europe, North Africa, and the Middle East. A Photographic Guide*. Bloomsbury Wildlife, London, United Kingdom. 547 p.
- Gvoždík V, Moravec J, Klütsch C, Kotlík P. 2010. Phylogeography of the Middle Eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics and Evolution* 55: 1,146–1,166.
- Jeřábková L, Zavadil V. 2020. *Atlas rozšíření obojživelníků České republiky* [Distribution Atlas of Amphibians in the Czech Republic]. Agentura ochrany přírody a krajiny ČR, Praha, Czech Republic. 104 p.
- Jeřábková L, Krása A, Zavadil V, Mikátová B, Rozínek R. 2017. Červený seznam obojživelníků a plazů České republiky [Red list of amphibians and reptiles of the Czech Republic]. Pp. 83–106 In: *Červený seznam ohrožených druhů České Republiky. Obratlovci [Red List of Threatened Species of the Czech Republic. Vertebrates]*. *Příroda* 34. Editors, Chobot K, Němec M. Agentura ochrany přírody a krajiny ČR, Praha, Czech Republic. 182 p.
- Jeřábková L, Šandera M, Baláž V. 2019. Conservation and decline of European amphibians: the Czech Republic. Pp. 58–67 In: *Status of Conservation and Decline of Amphibians: Eastern Hemisphere, Part 5: Northern Europe*. Amphibian Biology, Volume 11. Editors, Heatwole H, Wilkinson JW. Pelagic Publishing, Exeter, United Kingdom. 108 p.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kuba F. 2019. [The rarest Czech anuran lives in the dump in Krnov]. Available: <https://www.denik.cz/regiony/nejvzacnejsi-ceska-zaba-zije-v-krnove-na-skladce-20190808.html> [Accessed: 11 November 2019]. [In Czech].
- Maštera J, Zavadil V, Dvořák J. 2015. *Vajíčka a larvy obojživelníků České republiky* [Eggs and Larvae of Amphibians of the Czech Republic]. Academia, Praha, Czech Republic. 179 p.
- Moravec J. 1994. *Atlas rozšíření obojživelníků v České republice* [Atlas of Czech Amphibians]. Národní muzeum, Praha, Czech Republic. 136 p.
- Moravec J. 2019. *Obojživelníci a Plazi České Republiky* [Amphibians and Reptiles of the Czech Republic]. Academia, Praha, Czech Republic. 461 p.
- Najbar B, Vlček P, Šuhaj J. 2011. New locality record for the Agile Frog (*Rana dalmatina*) from an Odra River meander in southern Poland. *Herpetology Notes* 4: 63–65.
- Profus P, Sura P. 2018. Ropucha paskówka *Epidalea calamita* (Laurenti, 1768). Pp. 54–56 In: *Atlas płazów i gadów Polski. Status – Rozmieszczenie – Ochrona* [Atlas of the Amphibians and Reptiles of Poland. Status – Distribution – Conservation]. Editors, Głowaciński Z, Sura P. Wydawnictwo Naukowe PWN, Warsaw, Poland. 233 p.
- Pruner L, Míka P. 1996. Seznam obcí a jejich částí v České republice s čísly mapových polí pro síťové mapování fauny [List of settlements in the Czech Republic with associated map field codes for faunistic grid mapping system]. *Klapalekiana* 32(Suppl): 1–175.
- Quitt E. 1971. *Mapa klimatických oblastí ČSSR* [Map of Climatic Regions of Czechoslovakia]. Kartografie, Prague, Czech Republic and Geografický Ústav ČSAV, Brno, Czech Republic.
- Rowe G, Harris DJ, Beebee TJC. 2006. Lusitania revisited: a phylogeographic analysis of the Natterjack Toad *Bufo calamita* across its entire biogeographical range. *Molecular Phylogenetics and Evolution* 39: 335–346.
- Šandera M, Maštera J, Mačát Z, Vojar J. 2017. Záchranný program pro ropuchu krátkonožou (*Epidalea calamita*) v České republice [The Natterjack Toad (*Epidalea calamita*) action plan in the Czech Republic]. Herpeta, Praha, Czech Republic. Available: http://www.herpeta.cz/pages/serve.php?file=1575975907_0_zp_ropucha-pdf.pdf [Accessed: 10 December 2019].
- Šiffner F. 2011. Obojživelníci a plazi Krnovska [Amphibians and reptiles of the Krnov region]. *Herpetologické Informace (The Journal of the Czech Herpetological Society)* 10(2): 14–20.
- Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet P-A, Crnobrnja Isailovic J, Denoël M, Ficetola GF, Gonçalves J, et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia* 35: 1–31.
- Sinsch U. 2009. *Bufo calamita* Laurenti, 1768 – Kreuzkröte. Pp. 339–413 In: *Handbuch der Reptilien und Amphibien Europas. Band 5/II: Froschlurche (Anura) II (Hylidae, Bufonidae)*. Editor, Grossenbacher K. Aula-Verlag, Wiebelsheim, Germany. 503 p.
- Sinsch U. 2017. Wie weit wandern Amphibien? Verhaltensbiologische und genetische Schätzung der Konnektivität zwischen Lokalpopulationen. *Zeitschrift für Feldherpetologie* 24: 1–18.
- Speybroeck J, Beukema W, Bok B, Van Der Voort J. 2016. *Field Guide to the Amphibians and Reptiles of Britain and Europe*. Bloomsbury Natural History,

- London, United Kingdom and New York, New York, USA. 432 p.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Strachinis I, Karagianni KM, Stanchev M, Stanchev N. 2019. No one ever noticed: first report of *Zootoca vivipara* (Lichtenstein, 1823) in Greece. *Herpetology Notes* 12: 53–56.
- Świerad J. 1998. Herpetofauna na Górnym Śląsku [Herpetofauna of the Upper Silesia]. *Przestrzeń i Wartości. Studia i materiały waloryzacji przestrzeni Górnego Śląska* 2: 51–65.
- Vitáček Z, Zavadil V. 1994. Ropucha krátkonožá – *Bufo calamita* Laurenti, 1768. Pp. 66–68 In: *Atlas rozšíření obojživelníků v České Republice* [Atlas of Czech Amphibians]. Editor, Moravec J. Národní Museum, Prague, Czech Republic. 136 p.
- Vlček P, Najbar B, Jablonski D. 2010. First records of the Dice Snake (*Natrix tessellata*) from the North-Eastern part of the Czech Republic and Poland. *Herpetology Notes* 3: 23–26.
- Zavadil V. 1994. Die historische und aktuelle Verbreitung der Kreuzkröte in der Tschechischen Republik mit Bemerkungen über ihre Biologie. *Berichte Landesamt Umweltschutz Sachsen-Anhalt* 14: 39–40.
- Zavadil V. 1996. Ropucha krátkonožá, *Bufo calamita* Laurentii, 1768. Historické a aktuální rozšíření v České republice, s poznámkami o její biologii a ochraně [The Natterjack Toad, *Bufo calamita* Laurentii, 1768. Historical and present distribution in the Czech Republic, with notes on its biology and conservation]. *Ochrana přírody* 51: 135–138.
- Zavadil V, Sádlo J, Vojar J. 2011. *Biotopy našich obojživelníků a jejich management. Metodika AOPK ČR* [Biotopes of Czech Amphibians and their Management]. Agentura ochrany přírody a krajiny ČR, Prague, Czech Republic. 178 p.
- Zeisset I, Beebee TJC. 2014. Drift rather than selection dominates MHC class II allelic diversity patterns at the biogeographical range scale in Natterjack Toads, *Bufo calamita*. *PLoS ONE* 9(6): e100176.



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Vít Zavadil is a zoologist in ENKI (Třeboň, Czech Republic), a company focusing on environmental protection, especially issues related to the water in landscapes and its biodiversity. Vít is the first author of the action plan for the Aesculapian Snake (*Zamenis longissimus*) in the Czech Republic, which he had been working on until 2018. He has also worked on proposals and the enforcement actions of Special Areas of Conservation for amphibians in the Czech Republic, as defined by the European Union's Habitats Directive. In the last 25 years, he has primarily been studying biotopes after the mining of coal, minerals, and other construction materials, and the colonization of these habitats by vertebrates.



Václav Gvoždík is a herpetologist based at the Institute of Vertebrate Biology of the Czech Academy of Sciences (Brno, Czech Republic) and the National Museum (Prague, Czech Republic). Václav is interested in the phylogeography, diversity, and evolution of amphibians and reptiles of the Western Palearctic and tropical Africa. In the Western Palearctic, he is particularly experienced with the herpetofauna of Central and South-Eastern Europe, and the Middle East. He has special interests in the evolutionary biology of tree frogs (*Hyla*) and slow-worm lizards (*Anguis*).

Note: In 2020, after the acceptance of this article, another population of the Natterjack Toad (*Epidalea calamita*) was discovered by the authors and Filip Šiffner near Osoblaha in Czech Silesia (50.2624°N, 17.6922°E, 270 m asl), 21 km to the north from the Krnov-Cvilín locality (by air). At the Krnov-Cvilín site, the Natterjack Toad successfully bred in a naturally-formed puddle in June–July, laying eggs after heavy rains, and larvae metamorphosed in approximately three weeks. The artificial pond served as a successful breeding site for *Bombina variegata*, a Critically Endangered species in the Czech Republic.



Book Review

The Wildlife Techniques Manual, Eighth Edition

Howard O. Clark, Jr.

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Keywords. Capture techniques, climate change, conservation genetics, experimental design, population estimation, telemetry, unmanned aerial vehicle, urban wildlife management, vegetation analysis, wildlife damage management

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The 8th edition of *The Wildlife Techniques Manual* (Fig. 1) is a welcome sight in today's information hungry world. Since 1960, The Wildlife Society has produced several editions of techniques manuals that started off fairly modest, but now, in 2020, have grown into a monstrous, two-volume set (Fig. 2).

The chapters in the new manual are divided into two major categories: Research (Volume 1) and Management (Volume 2). The research volume is sub-divided into several sections, including Design and Analytical Techniques (7 chapters), Identification and Marking Techniques (4 chapters), Measuring Animal Abundance (7 chapters), Measuring Wildlife Habitat (4 chapters), and Research on Individual Animals (3 chapters). The management volume is divided into three sections: Management Perspectives (6 chapters), Managing Landscapes for Wildlife (12 chapters), and Managing Wildlife Populations (7 chapters). See the **Appendix** for a complete list of chapter titles and authors.

The 7th edition, which I reviewed in 2012 (Clark 2012), was the first time that the manual was published as a two volume set. The 8th edition continues this trend, but adds several new chapters; the 7th edition only had 37 chapters and the new edition has now grown to 50 chapters. As I predicted in 2012, the 8th edition reflects new challenges and research frontiers as wildlife managers and biologists invent new ways to study wildlife questions.

One of the most exciting and innovative approaches is explored in chapter 17: *Use of Unmanned Aerial Vehicles in Wildlife Ecology* (Rosario et al. 2020). The use of unmanned “drones” has exploded on the wildlife scene over the past few years. Drones are useful in capturing data on research subjects difficult to access via foot or vehicle. But one major caveat in using these drones is the Federal



Fig. 1. *The Wildlife Techniques Manual*, 2 Volumes. Editor, Silvy NJ. The Johns Hopkins University Press, Baltimore, Maryland, USA. 8th Edition, published 28 July 2020.

Trim Size: 8.5” × 11” | 1400 pages | Illustrations: 260 halftones, 165 line drawings | ISBN: 9781421436692 | Hardcover: US \$174.95. *Photo by Howard Clark.*

Aviation Administration's (FAA) Unmanned Aerial Vehicle (UAV) licensing and flight regulations. Safety is paramount when using drones and it is imperative that when using drones, wildlife managers and researchers understand the latest laws, directives, and policies. With a high level of FAA regulation understanding, better conservation of biological resources will result as well as an enriched research deliverable. The chapter covers several other topics, including types of UAV platforms and considerations, data management and analysis, UAVs in wildlife ecological research, and UAV safety. I was pleased to see a chapter on drones added to the 8th

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Fig. 2. *The Wildlife Techniques Manual* (8th edition, 2 volumes) compared to the slender 1st edition published 60 years earlier (Mosby 1960), which has 17 chapters. Photo by Howard Clark.

edition and I am sure as drone technology improves a chapter on UAVs continue to appear in future editions.

The final chapter, Chapter 50, *Managing Wildlife in a Changing Climate* (Inkley and Stein 2020), really binds all the others together. Although climate change (formerly known as “global warming”) has been on the scientific radar for decades (e.g., Chamberlin 1899) only now has a chapter in the manual been devoted to it. All of the research techniques and management philosophies discussed at length in *The Wildlife Techniques Manual* will be conducted under the auspices of global climate change. The trends of increased change in global temperatures (Fig. 3) have a significant effect on the global landscape and the wildlife species that occupy it. Research conducted from now on will no doubt have climate change as a factor, or at least something running in the background driving evolution and environmental adaptation. Chapter 50 provides an excellent overview and summary of the effects of climate change on wildlife. As the authors state on page 443, “The scientific record conclusively demonstrates that impacts of climate

change on wildlife are not just a concern for the distant future, but already are happening.” Climate effects are physically visible, such as the 17 of the 18 hottest years in the 136-year record have all occurred since 2001. We are witnessing catastrophic wildfires, hurricanes, droughts, and other extreme (but increasingly frequent) weather events. As noted in recent news media, the droughts in the western USA have driven beetle-kills of trees in western coniferous forests, which exacerbate the wildfire season. The “cause and effect” and interconnectedness of global climate change and landscape impacts are alarming.

In addition, Chapter 50 covers climate change basics, such as climate versus weather, climate models, scenarios of greenhouse gas concentrations, and best practices for the use of climate projections. An important section of the chapter covers abiotic and physical climate impacts, with discussions on elevated carbon dioxide levels, temperature changes, precipitation changes, intensified hurricanes and storms, snow cover changes, permafrost melting, declines in ice cover and glaciers, sea-level rise, ocean temperature increases, and ocean acidification. These sections paint a bleak picture, but subsequent sections provide approaches to mitigate the pending deleterious trends. The authors explore four overarching principles for effective climate adaptation:

1. Act with intentionality; link actions to climate impacts.
2. Manage for change, not just persistence.
3. Reconsider goals, not just strategies.
4. Integrate adaptation into existing work.

There are various things that we can do to respond to climate change, such as developments in wind energy and biofuel, changes in agricultural practices, shifting human population centers and infrastructure, and coastal armoring.

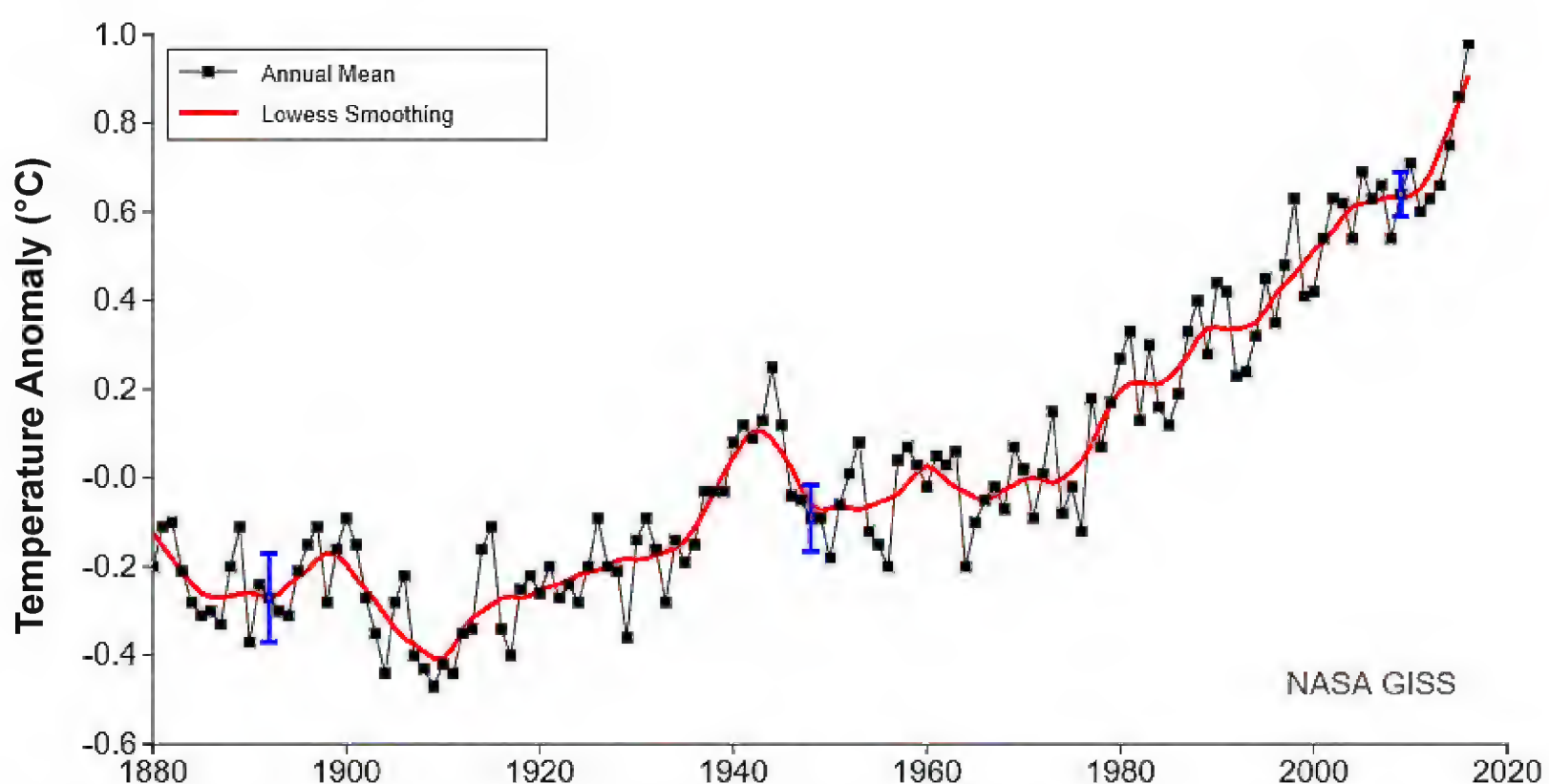


Fig. 3. Global temperature trends 1880–2017. Global mean estimates based on land and ocean data. <https://data.giss.nasa.gov/gistemp/graphs/>. Graphic in the Public Domain.

Chapter 50 is key in understanding global climate change and how we, as a species, can address and mitigate it. The authors state on page 468, “The future of our wildlife depends on wildlife professionals incorporating climate considerations into all aspects of their work.”

Overall, *The Wildlife Techniques Manual* is a critically important tool in the continued management and conservation of wildlife and landscape habitats. I encourage biologists and wildlife managers to field test the recommendations and guidance provided by the many authors who contributed to these monumental volumes. By working together, and using sound science, we may be able to create a sustainable global community on every level, launching us into a future of hope.

Acknowledgments.—I thank C.J. Randel and N.J. Silvy for allowing me to be a voice and participate in this extraordinary work. I am also incredibly grateful for the Johns Hopkins University Press production team and their collaboration effort with The Wildlife Society.

Literature Cited

Chamberlin TC. 1899. An attempt to frame a working hypothesis of the cause of glacial periods on an atmospheric basis. *The Journal of Geology* 7(6): 545–584.

Clark HO Jr. 2012. Book review of *The Wildlife Techniques Manual*. *Amphibian & Reptile Conservation* 5(1): 105–107 (e47).

Inkley DB, Stein BA. 2020. Managing wildlife in a changing climate. Pp. 443–470 In: *The Wildlife Techniques Manual*. Volume 2. 8th Edition. Editor, Silvy NJ. The Johns Hopkins University Press, Baltimore, Maryland, USA. 614 p.

Mosby HS. (Editor). 1960. *Manual of Game Investigational Techniques*. Edward Brothers, Inc., Ann Arbor, Michigan, USA. 364 p.

Rosario RG, Clayton MK, Gates IT. 2020. Use of unmanned aerial vehicles in wildlife ecology. Pp. 387–394 In: *The Wildlife Techniques Manual*. Volume 1. 8th Edition. Editor, Silvy NJ. The Johns Hopkins University Press, Baltimore, Maryland, USA. 759 p.

Appendix. The Wildlife Techniques Manual (8th edition, 2 volumes) list of chapters and authors.

Volume 1. Research		
List of Contributors		
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Design and Analytical Techniques		
Chapter 1	Research and Experimental Design	EO Garton, JL Aycrigg, C Conway, and JS Horne
Chapter 2	Management and Analysis of Wildlife Ecology Data	BA Collier and TW Schwertner
	Capturing and Handling Techniques	
Chapter 3	Capturing and Handling Wild Animals	NJ Silvy, RR Lopez, and TA Catanach
Chapter 4	Chemical Immobilization of Wildlife	ML Drew
Chapter 5	Use of Dogs in Wildlife Research and Management	DK Dahlgren, RD Elmore, DA (Smith) Woollett, A Hurt, JK Young, D Kinka, EB Arnett, D Baines, and JW Connelly
Chapter 6	Identifying and Handling Contaminant-Related Wildlife Mortality/Morbidity	SR Sheffield, JP Sullivan, and EF Hill
Chapter 7	Wildlife Health and Disease Surveillance, Investigation, and Management	MJ Peterson and PJ Ferro
Identification and Marking Techniques		
Chapter 8	Criteria for Sex and Age of Birds and Mammals	EK Lyons, MA Schroeder, and LA Robb
Chapter 9	Identification of Animals from Field Signs	JM Tomeček and J Evans
Chapter 10	Techniques of Marking Wildlife	NJ Silvy, RR Lopez, and MJ Peterson
Chapter 11	Radiotelemetry, Remote Monitoring, and Data Analyses	NJ Silvy and TA Catanach
Measuring Animal Abundance		
Chapter 12	Estimating Animal Abundance	BL Pierce, RR Lopez, and NJ Silvy
Chapter 13	Use of Remote Cameras in Wildlife Ecology	ID Parker, RR Lopez, and SL Locke
Chapter 14	Population Analysis in Wildlife Ecology	DH Johnson and SJ Dinsmore
Chapter 15	Use of Bioacoustics Monitoring Systems in Wildlife Research	JM Szewczak and ML Morrison
Chapter 16	Tracking Wildlife with Radar Techniques	TA Catanach and NJ Silvy
Chapter 17	Use of Unmanned Aerial Vehicles in Wildlife Ecology	RG Rosario, MK Clayton, and IT Gates
Chapter 18	Invertebrate Sampling Methods for Use in Wildlife Research	TA Catanach

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Chapter 21	Simulation Modeling in Wildlife Research	H-H (Rose) Wang and WE Grant
Chapter 22	Using Geospatial Technologies in Wildlife Studies	HL Perotto-Baldivieso, S Tapaneeyakul, and ZJ Pearson
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Chapter 27	Ethics in Wildlife Science and Conservation	MJ Peterson, MN Peterson, TR Peterson, and E von Essen
Chapter 28	Human Dimensions of Wildlife Management	SL Rodriguez and MN Peterson
Chapter 29	Communications and Outreach	SK Jacobson, HO Brown, and BS Lowe
Chapter 30	Conflict in Wildlife Science and Conservation	AM Feldpausch-Parker and TR Peterson
Chapter 31	Adaptive Management in Wildlife Conservation	JF Organ, DJ Decker, SJ Riley, JE McDonald, Jr., and SP Mahoney
Managing Landscapes for Wildlife		
Chapter 32	Forest Management for Wildlife	SW Bigelow, CG Mahan, AD Rodewald, LM Conner, and LL Smith
Chapter 33	Managing Rangelands for Wildlife	VC Bleich, MW Oehler, and JG Kie
Chapter 34	Managing Inland Wetlands for Wildlife	MK Laubhan, SL King, and LH Fredrickson
Chapter 35	Management of Coastal Wetlands for Wildlife	JA Nyman, C Elphick, and G Shriver
Chapter 36	Managing Farmlands for Wildlife	RE Warner, JW Walk, and JR Herkert
Chapter 37	Management and Research of Wildlife in Urban Environments	RA McCleery, CE Moorman, MC Wallace, and D Drake
Chapter 38	Managing Surface Disturbed Lands for Wildlife	TA Catanach and NJ Silvy
Chapter 39	Managing Disturbances to Wildlife and Habitats	CJ Parent, F Hernandez, and A Bruno
Chapter 40	Managing State Lands for Wildlife	TJ Ryder and JF Organ
Chapter 41	Managing Federal Lands for Wildlife	B Beard, RP Bixler, T Darden, B Huffaker, M Madison, and JG Van Ness
Chapter 42	Managing North American Indigenous Peoples' Wildlife Resources	H Stricker, PM Schmidt, J Gilbert, J Dau, DL Doan-Crider, S Hoagland, MT Kohl, CA Perez, LJ Van Daele, MB Van Daele, and D Dupont
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Chapter 46	Managing Terrestrial Invasive Species	TE Fulbright and TA Campbell
Chapter 47	Ecology and Management of Small Populations	JS Horne, LS Mills, JM Scott, KM Strickler, and SA Temple
Chapter 48	Captive Propagation and Translocation	D Drake and SA Temple
Chapter 49	Environmental Impact Assessment and Habitat Conservation Plans	CJ Randel, III, HO Clark, Jr., DP Newman, and TP Dixon
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A new species of *Lycodon* (Serpentes: Colubridae) from the Deccan Plateau of India, with notes on the range of *Lycodon travancoricus* (Beddome, 1870) and a revised key to peninsular Indian forms

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Abstract.—A new species of wolf snake, *Lycodon deccanensis* sp. nov., is described from southern India, from the hill ranges situated in the Deccan Plateau adjacent to the Southern Eastern Ghats and the Mysore uplands. The new species somewhat resembles, and has previously been confused with, another predominantly wet-zone taxon *Lycodon travancoricus*. The new species can be diagnosed by the following combination of characters: dorsal scale rows 16–17:17:15; usually 9 supralabials; ventrals 181–201; subcaudals 68–74, divided; an undivided anal scale; loreal in contact with internasal; nasal not in contact with prefrontal, separated by loreal-internasal contact; supraocular usually contacting prefrontal; preocular usually not contacting frontal; and a dorsum that is brownish in adults and blackish in juveniles, with white cross bars. Some previous records of *Lycodon travancoricus* (sic) from outside the Western Ghats represented the new species, while others were re-identified as *L. aulicus* and *L. anamallensis*, effectively restricting the range of *L. travancoricus* to the Western Ghats and Southern Eastern Ghats.

Keywords. Coloration, Deccan plateau, *Lycodon deccanensis* sp. nov., Reptilia, scalation, South Arcot, Tumkur

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Introduction

The Colubrid snake genus, *Lycodon* H. Boie in Schlegel, 1826, is a diverse group of non-venomous, nocturnal snakes inhabiting tropical Asia (Wallach et al. 2014; Uetz et al. 2020). In several parts of its vast range, which stretches from Transcaspia in the northwest to Sulawesi in the southeast (Wallach et al. 2014), many new species of *Lycodon* have been described in recent years (Grismer et al. 2014; Wostl et al. 2017; Jassen et al. 2019; Vogel and David 2019; Luu et al. 2019, 2020). The generic taxonomy of this group of snakes has been in a state of flux, as some authors (Guo et al. 2013; Siler et al. 2013) have included taxa traditionally (Smith 1943) classified under the genera *Dryocalamus* Günther, 1858

and *Dinodon* Duméril and Bibron, 1853. In the Indian peninsula (the elevated, triangular peninsular shield south of Vindhya, see Radhakrishna 1993), six species are currently known (Whitaker and Captain 2008; Aengals et al. 2018), namely: *Lycodon aulicus* (Linnaeus, 1758), *L. striatus* (Shaw, 1802), *L. anamallensis* Günther, 1864, *L. travancoricus* (Beddome, 1870), *L. flavomaculatus* Wall, 1908, and *L. flavicollis* Mukherjee and Bhupathy, 2007 (Smith 1943; Daniel 2002; Das 2002; Whitaker and Captain 2008; Ganesh and Vogel 2018).

The taxonomy and distribution of *Lycodon* species in South Asia still remain incompletely known. Based on a phylogenetic study, Pyron et al. (2013) clarified the affinities and generic allocation of the Sri Lankan species *L. carinatus* (Kuhl, 1820), which was previously

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regarded as the sole member of the genus *Cercaspis*. Ganesh and Vogel (2018) reassessed the taxonomy of one of the most ‘well-known’ and ‘common’ species, *L. aulicus*, and recognized *L. anamallensis* Günther, 1864 as valid, with the purportedly Sri Lankan endemic taxon *L. osmanhilli* Taylor, 1950 being its synonym. The distribution of *L. mackinnoni* Wall, 1906 in Pakistan was reported by Jablonski et al. (2019). Relating to this work, *L. travancoricus*, a species endemic to the hills of peninsular India, was recently redescribed and some incorrect identifications that have caused dubious extralimital localities in places such as Sindh, Pakistan, were also revealed (Ganesh et al. 2020a).

Within the Indian peninsula, certain geographical outlier records were known, such as those from near South Arcot district (Tamil Nadu) and Vizagpatnam (Andhra Pradesh) in the Eastern Ghats and from Jabulpore, near the Seoni Hills of Central India (Smith 1943). These were in fact historical reports of specimens identified as *L. travancoricus* in the collections of Zoological Survey of India - ZSI (Sclater 1891) and Bombay Natural History Society Museum - BNHS (Wall 1923). Recently, there has also been a report of an unidentified species of wolf snake, represented as *Lycodon* sp., from the Southern Eastern Ghats (Ganesh et al. 2018). While dealing with the catalogue of herpetological specimens in Sálím Ali Centre for Ornithology and Natural History (SACON) [Ganesh et al. 2020b], a damaged specimen (SACON/VR-93) of this species was listed as *Lycodon* sp. Our examination of the specimens identified as *L. travancoricus* from extralimital localities (i.e., outside the Western Ghats) indicated that these were not conspecific with *L. travancoricus*. While the specimens reported from the Northern Eastern Ghats and Central India represent *L. aulicus* and *L. anamallensis* (see below), the South Arcot specimen represents the undescribed species reported by Ganesh et al. (2018, 2020b) as *Lycodon* sp. A fresh collection of a dead-on-road specimen from the Bangalore uplands further indicates the conspecificity of these specimens. This innominate form is herein described as a new species.

Materials and Methods

A total of nine specimens representing this species, both preserved and live, were examined for this study, in addition to 95 specimens representing six regional congeners (Appendix 1). Seven uncollected specimens of the new species (four live and three dead), consisting of three juveniles and four adults, were also considered and included as referred non-type specimens. For this study we follow the definitions of the genus *Lycodon* as per Smith (1943) and Wickramasinghe et al. (2020), and we retained standard morphological characters used for *Lycodon* (also see Ganesh and Vogel 2018; Ganesh et al. 2020a). The pale bands on the body and tail were counted on one side, usually the right side when not damaged.

Minimally visible or incomplete bands were counted as one band; bands that were fused (often forming an “X”) were counted as two. Ventral plate counting followed Dowling (1951), and the subcaudals count exempted the terminal scale. Measurements, except body and tail lengths, were taken with a slide-caliper to the nearest 0.1 mm; all body measurements were made to the nearest millimeter. The dorsal scale rows were counted at one head length behind the head, at midbody (i.e., at the level of the ventral plate corresponding to one-half of the total number of ventrals), and at one head length before the vent. Half-ventrals were counted as one. The first scale under the tail meeting its opposite was regarded as the first subcaudal. The collar on the neck was not counted, and bands covering the anal shield were added to the bands of the body. Sex of preserved specimens was determined by dissection of the ventral tail base, while that of live individuals was examined to the extent possible by gentle anal palpation (also see Ganesh and Vogel 2018). Geographic coordinates were recorded *in situ* using a handheld GPS on a WGS-84 map datum, or were sourced from GoogleEarth software, and are represented in decimal degrees rounded to three decimal places.

Abbreviations. Avg.: average; SVL: snout to vent length; Collections. – BMNH: The Natural History Museum, London, United Kingdom. – BNHS: Bombay Natural History Society Museum, Mumbai, India. – CAS: California Academy of Sciences Museum, San Francisco, California, USA. – CESS: Centre for Ecological Sciences (Snakes), Bangalore, India. – CSPT/S: Chennai Snake Park Museum, Chennai, India. – FMNH: Field Museum of Natural History, Chicago, Illinois, USA. – NMW: Naturhistorisches Museum Wien, Vienna, Austria. – MCZ: Museum of Comparative Zoology, Harvard, Massachusetts, USA. – MHNG: Muséum d’Histoire Naturelle, Geneva, Switzerland. – SACON: Sálím Ali Centre for Ornithology and Natural History, Coimbatore, India. – SMF: Naturmuseum Senckenberg, Frankfurt am Main, Germany. – UPZM: University of Peradeniya Zoology Museum, Peradeniya, Sri Lanka. – ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany. – ZMB: Zoologisches Museum Berlin, Germany. – ZSI: Zoological Survey of India, Kolkata, India.

Taxonomy

Lycodon deccanensis sp. nov.

Lycodon travancoricus (nec Beddome, 1870) – Sclater 1891 part.

Lycodon sp. – Ganesh et al. (2018, 2020b).

urn:lsid:zoobank.org:act:79E6BFD8-DD3A-4604-ACB2-22BB71B81E76

Holotype: BNHS 3596, coll. K.G. Punith and Ashok Kumar Mallik in June 2012.

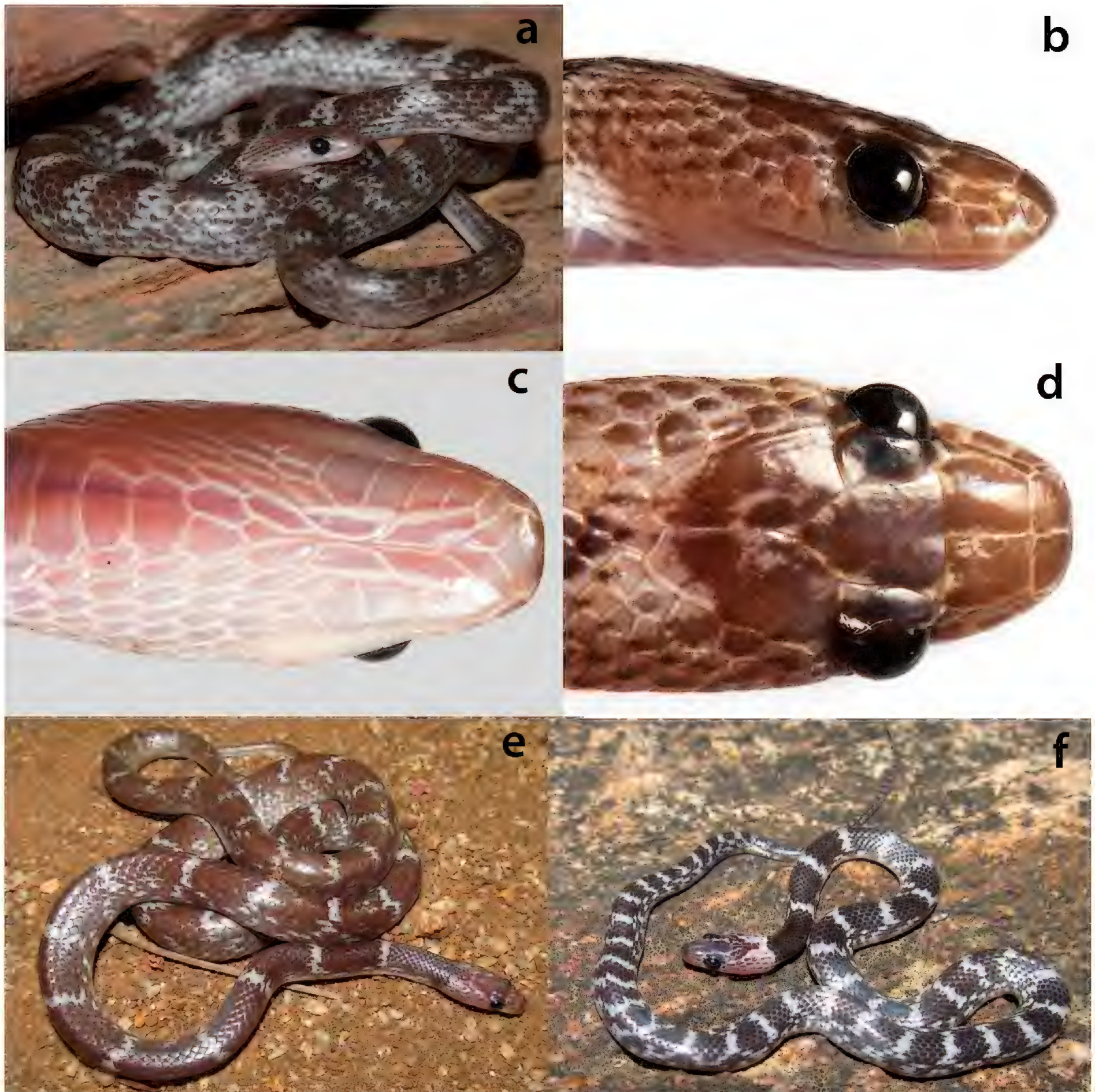


Fig. 1. *Lycodon deccanensis* sp. nov. in life: (a) entire, dorsolateral view; (b–d) head profiles of a live uncollected specimen from Devarayana Durga; (e–f) live uncollected adult and juvenile from Melagiri, showing ontogenic color shift. Photos by K.G. Punith, M.V. Shreeram, and S.R. Ganesh.

Type locality: Devarayana Durga (13.371°N, 77.210°E; 1,060 m asl) in Tumkur district, Karnataka, India.

Paratype: ZSI 13271 from South Arcot district, Tamil Nadu, India; Mus. Coll. Jaffa (also see Sclater 1891).

Referred specimens ($n = 7$): SACON/VR-93, a damaged specimen from Anaikatti, Coimbatore district, Tamil Nadu; two uncollected roadkill specimens sighted in 2011 in Bodha Malai, Salem-Namakkal districts, Tamil Nadu; two live individuals sighted in 2016 in Guthirayan hills, Krishnagiri district, Tamil Nadu, one live specimen sighted in Snamavu R.F. Hosur, Tamil Nadu, and one roadkill sighted in 2017 in Tirupati and Horsley Hills, Chittoor district, Andhra Pradesh.

Etymology: Toponym, named after its region of occurrence – the Deccan plateau, a raised table land of late Cretaceous origin, situated between the Eastern Ghats and the Western Ghats of the Indian peninsula.

Diagnosis: A species of *Lycodon* inhabiting the Deccan plateau of India, characterized by: small size (total length < 470 mm); scales smooth, in 16–17:17:15 rows, without apical pits; usually 9 supralabials (10, in one case); ventrals 181–201 ($n = 9$) angulate laterally; anal plate undivided; subcaudals 68–78 (84; $n = 8$), paired; loreal in contact with internasal, separate from eye; nasal not in contact with prefrontal; anterior pair of genials subequal to posterior pair; supraocular usually contacting prefrontal; preocular usually not contacting



Fig. 2. *Lycodon deccanensis* sp. nov. in preservative: (a–b) entire and head closeup (inset) of Paratype ZSI 13271; (c) entire view of SACON/VR-93. Photos by K. Deuti and S.R.Ganesh.

frontal (preocular separating frontal, prefrontal, and supraocular in one case); dorsum brown in adults and black in juveniles, with white cross bars.

Due to the slender body and smaller head, the new species superficially resembles the genus *Dryocalamus*, its higher midbody scale rows (17) and lower ventral counts (181–201; avg. 190; $n = 9$) [vs. rows 13–15; ventrals 200+ in *Dryocalamus*, see Smith 1943] clearly indicate this species belongs to the genus *Lycodon*, even if *Dryocalamus* is regarded as a valid genus.

Description of the Holotype

Measurements (all in mm): Snout-vent length 212; tail length: 31+? (tail cut); head length: 8.2; head width: 5.8;

eye-snout distance: 2.6; eye diameter: 1.9; internarial distance: 2.4; interocular distance: 3.5; mandible-eye distance: 6.2.

Habitus: Body rather slender and elongate; head slightly distinct from neck; tail fairly long and tapered; head flat and depressed, not quite spatulate; posterior temporal regions not distinctly bulbous and enlarged; ventrolateral region with a grooved margin; canthus rostralis not well-defined; snout oblong to rounded in lateral view.

Scalation: Scales smooth, without apical pits; dorsal scale rows: 16, 17, 15 rostral visible from above, contacting nasals; supralabials: 9 (3–5 touching eye); infralabials: 9 (1–5 touching anterior genial); ventrals 198 (angulate laterally); anal plate entire; paired subcaudals 28+? (tail cut).

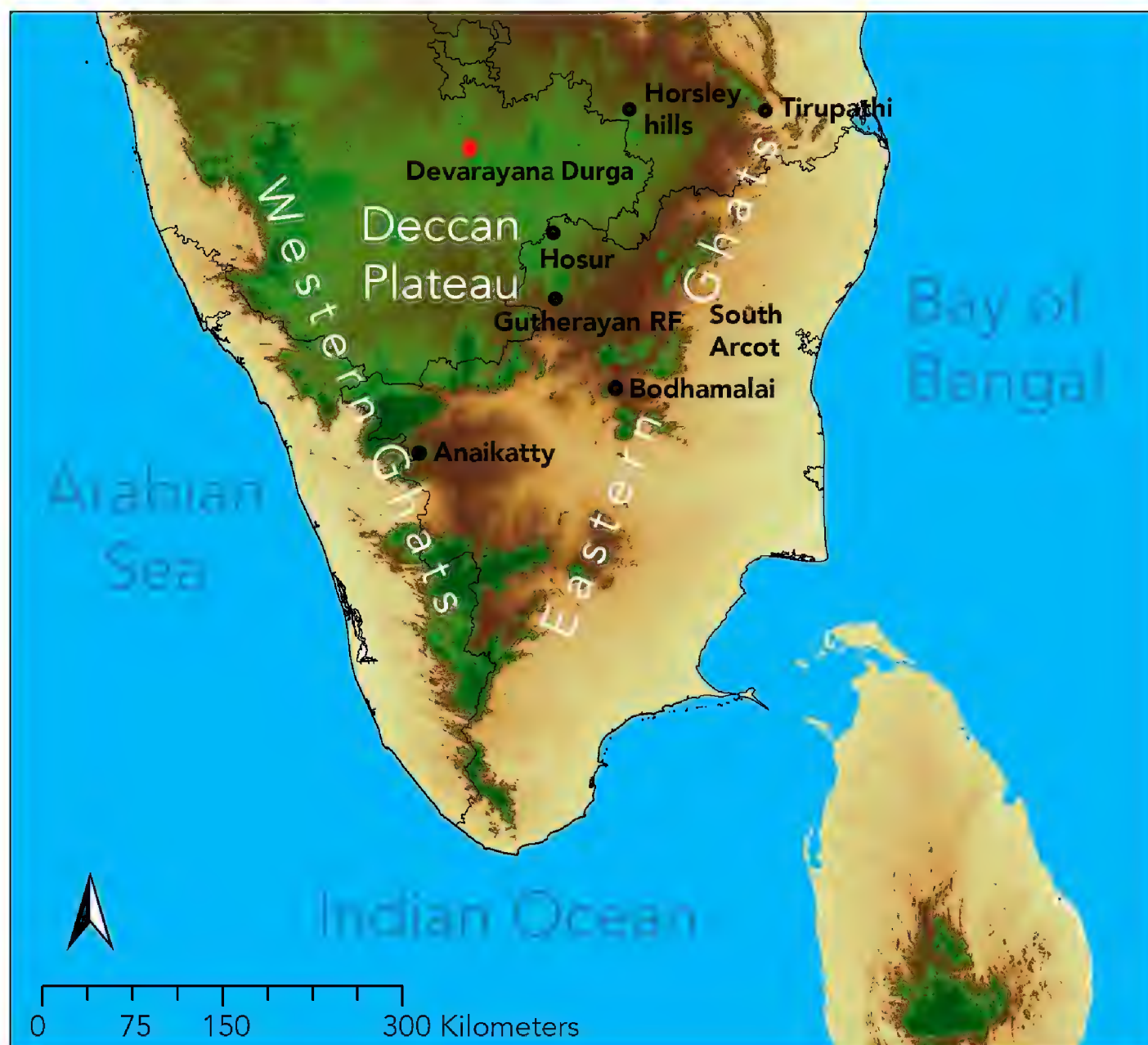


Fig. 3. Map showing the type locality and distribution records of *Lycodon deccanensis* sp. nov. Type locality (Devarayana Durga) marked with a red dot.

Coloration in life: Dorsum deep brown with 48 white cross bars on body; cross bars present vertebally, not extending to full circumference of body along the flanks, wider anteriorly and narrower posteriorly, much thinner and well-spaced anteriorly, thicker and close-set posteriorly; lateral sides of body with white squared spots either between two or subsequent to vertebral cross bars, giving it an overall white-mottled appearance; a distinctive white wash covering the whole posterior part of head from postocular, temporal regions encapsulating until parietal and occipital regions; almost all scales on head presenting a distinctive white outline, except the frontal and prefrontal parts that may have white flecks inside.

Coloration in preservation: After preservation in alcohol for eight years, dark brownish ground color much faded in intensity to light creamy brown; contrasting white barred pattern less evident; eyes cloudy white.

Variation: In agreement with the holotype in most respects, and showing the following intraspecific variation (paratype): ventrals 181, subcaudals 72 pairs; 52 white cross bars on body; preocular separating frontal, prefrontal, and supraocular; measurements in mm: snout-vent length: 168; tail length: 42.50; head length: 7.18; head width: 5.19; eye-snout distance: 2.79; eye diameter: 1.55; internarial distance: 2.04; interocular distance:

3.10; inferior eye margin to upper lip margin distance: 0.74; the damaged specimen SACON/VR-93 has parts of head missing, 188 ventrals, 64 paired subcaudals, 49 white cross bars on body and measurements (mm): snout-vent length: 280; tail length: 60; body width: 6.35.

The live individuals were very similar to the holotype in morphology, and show the following variation: infralabials 10 or 11 on either side; body scales in 17:17:15 rows, all smooth and glossy; ventrals 181–201, notched laterally; anal plate undivided; subcaudals 68–78 (84 outlier value) pairs. Adults (total length 360–450 mm) much more brownish; whereas juveniles (< 200 mm) dark coffee-brown to pitch black ground color, on which the white cross bars appear as usual.

Distribution and natural history. Based on the specimens observed *in situ* during fieldwork, this species appears to inhabit mid- to higher elevations (> 600 m asl), and hilly forest tracts in the Deccan plateau, such as the taller isolated peaks in the Eastern Ghats and the Mysore uplands. The two examined specimens in museum collections (ZSI and SACON), come from near South Arcot (ca. 11.77°N, 78.75°E; 850 m asl) and Anaikatti (11.092°N, 76.778°E; 670 m asl), respectively. Though the exact place names given on the jar labels of these specimens furnish coarse-level geographic data, the places are always associated with the presence of hills nearby (see Ganesh et al. 2018), attesting to its affinity for the hills.



Fig. 4. Extralimital records re-identified as (a–b) *Lycopodon anamallensis* (BNHS 1602a and b) and (c–e) *Lycopodon* cf. *aulicus* (BNHS 1603 and 1604). Photos by A. Omkar.

Like most members of the genus, this species is usually nocturnal, as the four active individuals were sighted at night during fieldwork. At least the juveniles are semi-arboreal, and have been seen twice climbing trees and building walls, similar to the habits of some *Lycopodon* species and especially *Dryocalamus* species (Smith 1943). Potential prey species (pers. obs. in other *Lycopodon* species) recorded in the vicinity of these snakes are: *Cnemaspis graniticola* in Horsley Hills that were sleeping on the same building wall; and *Hemiphyllodactylus jnana* in Melagiri Hills, that were seen on plants near roadsides (also see Agarwal et al. 2019, 2020).

Regarding the observations on uncollected specimens seen *in situ* in August 2011, two juvenile roadkills were seen in Bodha Malai (11.543°N, 78.184°E; 920 m asl), in the Eastern Ghats (Namakkal district, Tamil Nadu). The surrounding area was dry evergreen forest, with a pliable tar road on which the dead snakes were noticed. Sympatric snakes noted were *Rhinophis goweri*, *Uropeltis rajendrani*, and *Naja naja*. In June 2016, at 2050 h, a juvenile was sighted at 1.3 m crawling over tree bark atop a dry evergreen forest patch in Guthirayan Hills (12.290°N, 77.837°E; 1,400 m asl) of Melagiris, in the

Eastern Ghats (Krishnagiri district, Tamil Nadu). After two days, at 2210 h, an adult was seen on bare ground bordering a road in the same forest area. This area was covered with semi-evergreen forests. Sympatric snakes sighted were *Uropeltis* cf. *elliotti*, *Trimeresurus gramineus*, *Dendrelaphis tristis*, *Boiga trigonata*, *B. flaviviridis*, and *B. nuchalis* (also see Ganesh et al. 2018). In July 2016, an adult individual was found on the road near Sanamavu RF (12.665°N, 77.874°E; 800 m asl), Hosur, with a surrounding habitat similar to the type locality, dominated by dry forests and eucalyptus plantations. In June 2017, a juvenile was sighted at 1945 h, crawling at a height of 1.5 m on the walls of an old, abandoned building in Horsley Hills (13.650°N, 78.393°E; 1,200 m asl), a part of the Mysore plateau (Chittoor district, Andhra Pradesh). The vegetation in the vicinity was rather anthropogenically-modified, with dry evergreen forests intermixed with eucalyptus plantations. Sympatric snakes sighted were *Lycopodon flavicollis*, *Oligodon taeniolatus*, *Coelognathus* cf. *helena* complex, and *Bungarus caeruleus* (the latter as roadkill). In September 2017, an adult roadkill *Lycopodon deccanensis* **sp. nov.** was found in the Tirupati hills (13.683°N, 79.357°E; 900 m asl). The surrounding habitat

Table 1. Identities and morphological features of specimens erroneously reported in the literature as *Lycodon travancoricus* (sic) from outside the Western Ghats (Cocanada or Kakinada, Vizagapatnam and Jabbulpore) apart from the paratype of *Lycodon deccanensis* **sp. nov.**

Species (here re-identified)	<i>Lycodon anamallensis</i>	<i>Lycodon anamallensis</i>	<i>Lycodon</i> cf. <i>aulicus</i>	<i>Lycodon</i> cf. <i>aulicus</i>
Museum Registration Number	BNHS 1062A	BNHS 1062B	BNHS 1063	BNHS 1064
Locality	Cocanada (=Kakinada)	Cocanada (=Kakinada)	Vizagapatnam, Madras Presidency	Jabbulpore, Central India
Scale rows	15:17:15	16:17:15	16:17:16	16:17:16
Supralabials	9/9	9/9	9/10	9/9
Infralabials	10/10	10/11	11/11	11/11
Temporals	10	11	10	10
Anal scale	Divided	Divided	Divided	Divided
Ventrals	195+3	197+3	195+3	194
Subcaudals	67 pairs	59 pairs	66 pairs	58 pairs
Loreal-internasal	Contacting	Contacting	Not contacting	Not contacting
Nasal-Prefrontal	Not contacting	Not contacting	Contacting	Contacting
Supraocular-prefrontal	Barely touching	Barely touching	Not contacting	Not contacting
Preocular-Frontal	Barely touching	Barely touching	Contacting	Contacting
Snout-vent length	402 mm	357 mm	449 mm	310 mm
Tail length	102 mm	74 mm	96 mm	62 mm

was dominated by dry and mixed deciduous forests, with active road traffic.

Comparisons: Here, *Lycodon deccanensis* **sp. nov.** is compared with all the known South Asian congeners (with only the opposing suite of character states listed). *Lycodon aulicus* (Linnaeus, 1758): anal plate undivided; supraocular not contacting prefrontal; preocular usually not contacting frontal. *Lycodon striatus* (Shaw, 1802): anal plate undivided; head not short and rounded; neck not indistinct; supralabials usually 9; higher ventral count (154–166 vs. 181–201 in new species); absence of yellow vertebral spots. *Lycodon anamallensis* Günther, 1864: anal plate undivided; white outlines in scales on top of posterior head, across parietals; dorsal cross bars white, never quite yellow; supralabials not distinctly creamy spotted with brown. *Lycodon travancoricus* (Beddome, 1870): subcaudals often undivided; loreal in contact with internasal; nasal not in contact with prefrontal; anterior genials subequal to posterior pair; supraocular usually contacting prefrontal; preocular usually not contacting frontal; *Lycodon flavomaculatus* Wall, 1908: anal plate undivided; higher ventral count (165–183 vs. 181–201 in new species); presence of distinct yellow vertebral spots. *Lycodon flavicollis* Mukherjee and Bhupathy, 2007: anal plate undivided; no distinct yellow collar mark; presence of white cross bars on dorsum, even in adults.

Identities of *Lycodon travancoricus* (sic) Records from Outside the Western Ghats

At least one report of *L. travancoricus* (sic), from ‘South Arcot’ (Sclater 1891) is relevant in the description of this

new species, *Lycodon deccanensis* **sp. nov.** Therefore, we also re-examined the specimens that are the basis of other such reports in the literature (Vizagapatnam and Jabbulpore: Wall 1923; Cocanada: Underwood 1947). Based on our re-examination (Fig. 4; Table 1), these records now represent *Lycodon* cf. *aulicus* (Vizagapatnam, Jabbulpore) and *L. anamallensis* (Cocanada). Our finding in turn restricts the distribution range of *L. travancoricus* to the Western Ghats (Ashambu to Surat Dangs) and the Southern Eastern Ghats (Sirumalai, Shevaroy, Kolli, and Bilgiri Hills).

Discussion

The finding of a new species of *Lycodon* from the semi-evergreen belts of the hill ranges constituting the Eastern Ghats and the Mysore uplands is not that surprising. In a regional sense, it is in keeping with other recent findings of new snakes from this region, e.g., *Lycodon flavicollis* by Mukherjee and Bhupathy (2007); *Boiga flaviviridis* by Vogel and Ganesh (2013); *Rhinophis goweri* by Aengals and Ganesh (2013); and *Uropeltis rajendrani* by Ganesh and Achyuthan (2020). As had already been highlighted (Agarwal et al. 2019, 2020; Ganesh et al. 2018), these hill ranges and elevated plateaus have not yet been systematically surveyed by herpetologists, especially for snakes.

This work is essentially an extension of Ganesh et al. (2020a), in that it further clarifies the supposedly extralimital records of “*L. travancoricus*” (sic), such as those for South Arcot (Sclater 1891), Cocanada (Underwood 1947), and Vizagapatnam and Jabbulpore (Wall 1923). Apart from this new species representing the

record of South Arcot, two more species *L. anamallensis* and *Lycodon* cf. *aulicus* were involved in the records of Cocanada as well as Vizagaptnam and Jabulpore, respectively (see Table 2). The fact that *L. anamallensis* represented incorrect records of *L. travancoricus* (sic), again supports a similar finding (Ganesh et al. 2020a) in the Museum of Comparative Zoology, USA. Thus, based on the outcomes of these studies, the distribution range of *Lycodon travancoricus* is here restricted to the Western Ghats and the Southern Eastern Ghats.

This new species *Lycodon deccanensis* **sp. nov.** has been known to the herpetological community for at least the past 125 years, since the time of Sclater (1891). That it was lurking under the wrong name (*L. travancoricus*) once again underscores the necessity of ensuring accurate taxonomy, as well as in reporting geographical (or morphological) outliers. Further research is recommended to document the total distribution range, as well as the natural history and basic biology, of this new species.

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Revised key to South Asian *Lycodon* species (modified from Ganesh and Vogel 2018)

- 1a. Body scales strongly keeled.....*L. carinatus*
- 1b. Body scales not strongly keeled.....2
- 2a. Anal plate entire.....3
- 2b. Anal plate divided.....4
- 3a. Loreal not contacting internasal; nasal contacting prefrontal.....*L. travancoricus*
- 3b. Loreal contacting internasal; nasal not contacting prefrontal.....*L. deccanensis* **sp. nov.**
- 4a. Ventrals < 200, body more black than brown.....5
- 4b. Ventrals > 200, body more brown than black.....6
- 5a. Usually 8 supralabials, reticulations white or with yellow mid-spots.....*L. striatus*
- 5b. Usually 9 supralabials, reticulations always yellow.....*L. flavomaculatus*
- 6a. Yellow collar always present, no other pattern, ventrals not angulate laterally.....*L. flavicollis*
- 6b. Collar present or absent, body uniform or banded, ventrals angulate laterally.....7
- 7a. Collar present, touching the parietals, converging towards snout tip.....*L. aulicus*
- 7b. Collar absent, first band far away from parietals, converging towards tail.....*L. anamallensis*

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Literature Cited

- Aengals R, Ganesh SR. 2013. *Rhinophis goweri*, a new species of Shieldtail Snake from the southern Eastern Ghats, India. *Russian Journal of Herpetology* 20(1): 61–65.
- Aengals R, Kumar VMS, Palot MJ, Ganesh SR. 2018. *A Checklist of Reptiles of India. Version 3.0*. Zoological Survey of India, Kolkata, India. 35 p.
- Agarwal I, Khandekar A, Giri VB, Ramakrishnan U, Karanth KP. 2019. The hills are alive with geckos! A radiation of a dozen species on sky islands across peninsular India (Squamata: Gekkonidae, *Hemiphyllodactylus*) with the description of three new species. *Organisms, Diversity, and Evolution* 19(2): 341–361.
- Agarwal I, Thackeray T, Pal S, Khandekar A. 2020. Granite boulders act as deep-time climate refugia: a Miocene divergent clade of rupicolous *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the Mysore Plateau, India, with descriptions of three new species. *Journal of Zoological Systematics and Evolutionary Research*. [In press]. <https://doi.org/10.1111/jzs.12391>
- Daniel JC. 2002. *The Book of Indian Reptiles and Amphibians*. Oxford Books, Oxford, United Kingdom and Bombay Natural History Society, Mumbai, India. 238 p.
- Das I. 2002. *A Photographic Guide to Snakes and other Reptiles of India*. New Holland, London, United Kingdom. 144 p.
- Dowling HG. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Ganesh SR, Vogel G. 2018. Taxonomic reassessment of the Common Indian Wolf Snakes *Lycodon aulicus* (Linnaeus, 1758) complex (Squamata: Serpentes: Colubridae). *Bonn Zoological Bulletin* 67(1): 25–36.

- Ganesh SR, Kalaimani A, Karthik P, Baskaran N, Nagarajan R, Chandramouli SR. 2018. Herpetofauna of Southern Eastern Ghats, India-II; from Western Ghats to Coromandel Coast. *Asian Journal of Conservation Biology* 7(1): 28–45.
- Ganesh SR, Amarasinghe AAT, Vogel G. 2020a. Redescription of *Lycodon travancoricus* (Beddome, 1870) (Reptilia: Colubridae), an Indian endemic snake, with a review of its geographic range. *Taprobanica* 9(1): 50–58.
- Ganesh SR, Bhupathy S, Karthik P, Rao GB, Babu S. 2020b. Catalogue of herpetological specimens at the Sálím Ali Centre for Ornithology and Natural History, India. *Journal of Threatened Taxa* 12(9): 16,123–16,135.
- Ganesh SR, Achyuthan NS. 2020. A new species of Shieldtail Snake (Reptilia: Squamata: Uropeltidae) from Kolli Hill complex, southern Eastern Ghats, peninsular India. *Journal of Threatened Taxa* 12(4): 15,436–15,442.
- Grismer LL, Quah ES, Anuar S, Muin MA, Wood Jr PL, Nor S. 2014. A diminutive new species of cave-dwelling Wolf Snake (Colubridae: *Lycodon* Boie, 1826) from Peninsular Malaysia. *Zootaxa* 3815(1): 51–67.
- Guo P, Zhang L, Liu Q, Li C, Pyron RA, Jiang K, Burbrink FT. 2013. *Lycodon* and *Dinodon*: one genus or two? Evidence from molecular phylogenetics and morphological comparisons. *Molecular Phylogenetics and Evolution* 68(1): 144–149.
- Jablonski D, Masroor R, Khan MA, Altaf M. 2019. Addition to the snake fauna of Pakistan: Mackinnon's Wolf Snake, *Lycodon mackinnoni* Wall, 1906. *Herpetological Bulletin* 147: 21–23.
- Janssen HY, Pham CT, Ngo HT, Le MD, Nguyen TQ, Ziegler T. 2019. A new species of *Lycodon* Boie, 1826 (Serpentes, Colubridae) from northern Vietnam. *ZooKeys* 875: 1–29.
- Luu VQ, Ziegler T, Ha NV, Le MD, Hoang TT. 2019. A new species of *Lycodon* Boie, 1826 (Serpentes: Colubridae) from Thanh Hoa Province, Vietnam. *Zootaxa* 4586(2): 261–277.
- Luu VQ, Bonkowski M, Nguyen TQ, Le MD, Calame T, Ziegler T. 2020. A new species of *Lycodon* Boie, 1826 (Serpentes: Colubridae) from central Laos. *Revue Suisse de Zoologie* 125(2): 263–276.
- Radhakrishna BP. 1993. Neogene uplift and geomorphic rejuvenation of the Indian Peninsula. *Current Science* 64: 787–793.
- Sclater WL. 1891. *List of Snakes in the Indian Museum*. Printed by the order of the Trustees of the Indian Museum, Calcutta, British India. 79 p.
- Siler CD, Oliveros CH, Santanen A, Brown RM. 2013. Multilocus phylogeny reveals unexpected diversification patterns in Asian Wolf Snakes (genus *Lycodon*). *Zoologica Scripta* 42(3): 262–277.
- Vogel G, David P. 2019. A new species of the *Lycodon fasciatus* complex from the Khorat Plateau, eastern Thailand (Reptiles, Squamata, Colubridae). *Zootaxa* 4577(3): 515–528.
- Vogel G, Ganesh SR. 2013. A new species of Cat Snake (Reptilia: Serpentes: Colubridae: *Boiga*) from dry forests of eastern Peninsular India. *Zootaxa* 3637(2): 158–168.
- Uetz P, Hallermann J, Hosek J. (Editors). 2020. The Reptile Database. Available: <http://reptile-database.reptarium.cz/> [Accessed: 1 June 2020].
- Underwood G. 1947. Reptiles of Cocanada. *Journal of the Bombay Natural History Society* 46: 613–628.
- Wall F. 1923. A hand-list of the snakes of the Indian Empire. *Journal of the Bombay Natural History Society* 29(2): 345–361.
- Wallach V, Williams KL, Boundy J. 2014. *Snakes of the World: a Catalogue of Living and Extinct Species*. CRC Press, Boca Raton, Florida, USA. 1,237 p.
- Whitaker R, Captain A. 2008. *Snakes of India – the Field Guide*. Draco Books, Chengalpat, India. 387 p.
- Wickramasinghe LM, Vidanapathirana DR, Pushpamal V, Wickramasinghe N. 2020. A new species of *Dryocalamus* (Serpentes: Colubridae) endemic to the rainforests of southwestern Sri Lanka. *Zootaxa* 4748(2): 248–260.
- Wostl E, Hamidy A, Kurniawa N, Smith EN. 2017. A new species of Wolf Snake of the genus *Lycodon* H. Boie in Fitzinger (Squamata: Colubridae) from the Aceh Province of northern Sumatra, Indonesia. *Zootaxa* 4276(4): 539–553.



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K.G. Punith is a business management student who runs the non-governmental organization WEROAR (Wild Animal Emancipation Reptile Oriented Awareness and Rescue). The organization is creating awareness about nature conservation among the Tumkuru (Karnataka, India) public and is also working towards the mitigation of human-wild animal conflicts in the same area.



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Gernot Vogel was born in Heidelberg, Germany, obtained his Ph.D. in Chemistry, and is now working as a chemist. Beginning as a reptile keeper, Gernot developed a great interest in the snake fauna of the Orient. His special interest lies in the systematics of snake genera with large distribution areas, such as *Trimeresurus*, *Boiga*, *Oligodon*, *Lycodon*, *Pareas*, *Dendrelaphis*, and others, with a primary geographical emphasis on China, India, and Indonesia.

Appendix 1. List of comparative material examined.

***Lycodon travancoricus*:** INDIA: BMNH 1946.1.13.75 (Syntype) Travancore, Attraymallay; CAS 15967, Ernakulam, Cochin State; ZSI 13695, ZSI 13696, Piermed (3,500 ft), Travancore, South India; ZSI 13396, Coonoor, Nilgiris; ZSI 17693, 'India,' no locality; FMNH 217705, Ponmudi, Trevandrum district, Kerala; ZSI 13694 and ZSI 13698; ZSI 13531, Koppa, Mysore; SACON/VR-97 Meghamalai, Theni district, Tamil Nadu; BNHS 1061 Tinnevely [= Tirunelveli district], Tamil Nadu; BNHS 1067, 1068, 1069, 1070 all from Matheran, Bombay Presidency; BNHS 1071 Mahabaleshwar, Western Ghats; BNHS 1072 Paralai, Valparai, Anamalai Hills; BNHS 2738 Shevaroy Hills, Madras Presidency; BNHS 2739 Khanapur, Belgaum district, Mysore State; BNHS 2740 Gersoppa Falls, Mysore State.

***Lycodon anamallensis*:** INDIA: BMNH 1946.1.14.92 (Holotype), Anamallays; BMNH 1904.10.18.2, Cannanore, Malabar, south India; BMNH 1904.10.18.4, Cannanore, Malabar, South India; CSPT/ S-28b, Madras; BMNH 1904.10.18.3 Cannanore, Malabar, Kerala; BMNH no number, Madras; BMNH 1924.10.13.7, Mundakayan, Travancore, Kerala; CSPT/S-28a, Madras; NMW 21707 Malabar; MCZ R2232 Pondicheri; SRI LANKA: FMNH 25927, from Colombo; MHNG 1198.70 Sri Lanka, no locality; ZFMK 32253, Sri Lanka, no locality; UPZM-17a and b, Peradeniya Kandy; MHNG 744.7, Ceylon [= Sri Lanka], no locality; NMW 21689.4, Ceylon [= Sri Lanka].

***Lycodon striatus*:** INDIA: BNHS 1083 Nilambur, Malabar; BNHS 1084, 1085 Madras; BNHS 1086 Secunderabad, Hyderabad; SACON/VR-96 Chinnamannur, Theni district, Tamil Nadu; SRI LANKA: ZFMK 52511, Kitulgala; ZFMK 52137, Kitulgala; ZFMK 52510, 'Sri Lanka,' no locality.

***Lycodon aulicus*:** MYANMAR: NMW 21699.1, Bhamo; CAS 215387, Sagaing; CAS 205000, DNA tested, Rakhin; CAS 245960, Tanintharyi; CAS 219800, Ayeyarwadi; NMW 14483, no locality; ZMB 11625, no locality; NMW 21702.2, Pegu; ZMB 10258, Minhla; BMNH 1928.1.4.1, Rangun; NEPAL: FMNH 62427, Tansing; BMNH 1936.7.2.2, Mae District, Doons; BMNH 80.11.10.138, 'Nepal' no locality; BMNH 1984.12.16, Royal Chitwan; FMNH 83090, Kathmandu; PAKISTAN: SMF 64484, Lahore, W-Pakistan; INDIA: BMNH 1908.5.23.15, Diburgash, Assam; FMNH 165108, Junganathpur, West Bengal; FMNH 8650, Central province near Chanda; FMNH 60647, Central province, Balaghat dist; BMNH 82.8.26.22, Kinelly (=Kimdey) hills, [Andhra Pradesh]; BMNH 74.4.29.958, Wynads, [Kerala]; ZMB 1790, Bengal; BMNH 1904.10.18.5, Cannanore, Malabar; NMW 37406:1, Ahmednagar, Maharashtra; NMW 37406:2, Ahmednagar, Maharashtra; CAS-SU 12263 Bistrampur, Madhya Pradesh; FMNH 165107 West Bengal, Howrah Dist.; FMNH 161469 West Bengal, Barnijunoh; NMW 14487.1 'Alakan,' ZMB 1791 Bengal; ZMB 9956 Ajmere, Rajasthan; ZMB 1806 Calcutta; NMW 14488 Kolkata; BMNH 1921.6.15.3 Bangalore, Karnataka; SMF 32463 Agra; ZMB 1791 Bengal; BMNH 1955.1.3.11 Mysore, 3,500 ft, Karnataka; BMNH 1936.1.3.4 Namakal, Tamil Nadu; BMNH 1924.10.13.9 Punakanaat, 700 ft, Travancore, Kerala; BMNH 69.8.28.94 Matheran, Maharashtra; MCZ R3877, R4783 Madras; SRI LANKA: FMNH 123906, Ceylon, no locality; ZFMK 52137, Kitulgala; ZFMK 52511, Kitulgala; NMW 21689:5-7, no locality; NMW 14487:2-3, no locality; FMNH 123907 Ceylon, Trincomalee; ZFMK 52510 Sri Lanka, no locality; NMW 21689:1-3 Sri Lanka, no locality; NMW 14487:1 Sri Lanka; INDIAN OCEAN ISLANDS: ZFMK 29976, Mauritius; ZMB 8158 Île Bourbon [=La Réunion]; ZFMK 21766 Mascarenes, Reunion, Manapany; ZFMK 29977 Mauritius.



Book Review

The Dangerous Snakes of Africa

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Keywords. Adder, antivenom, asp, boomslang, cobra, colubrid, elapid, mamba, python, snakebite, venom, viper

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Authors Stephen Spawls and the late Bill Branch (1946–2018; see Conradie et al. 2019) have produced a pivotal book, *The Dangerous Snakes of Africa* (Fig. 1). Worldwide, snakebite affects an estimated 4.5 million people annually, claiming 125,000 human lives. In Africa alone, it is estimated that between 80,000 and 420,000 people are bitten each year, resulting in anywhere between 3,500 and 30,000 fatalities. Impacts from snake bites are significant medical issues that need attention. *The Dangerous Snakes of Africa* is a step forward in addressing the need.

Following a field guide format, the book covers 137 dangerous snakes (both venomous and nonvenomous) as well as another 70 species that can be mistaken as dangerous (see Clark 2012). The Introduction is a must read; it provides the background information needed to fully appreciate the book. The Introduction is divided into five sections: (1) Africa's snakes: which ones are dangerous?, (2) Where are the dangerous snakes in Africa?, (3) Using the maps in this book, (4) A note on conservation, and (5) Identifying a snake. Section 5 is particularly pertinent because it provides the reader with the tools needed to distinguish snakes from other reptiles. Included are diagrams showing head scales from various views, how to tell keeled and unkeeled scales apart, how to tell snake tail cloacal and subcaudal scales apart, and how to count the dorsal scale rows of a snake. Another important part of the introduction is identification strategies for living snakes that may quickly disappear. Often, observers only have a few seconds to view a snake and identification may be difficult. Key points to record include an estimate of the snake's size, shape, and appearance; take careful note of the color and distinctive markings or patterns; note its thickness (pencil vs. broomstick, or larger); and behavior—was it on the ground, in a tree, did it move quickly or slowly, hiss or strike? If a dead snake is encountered, then

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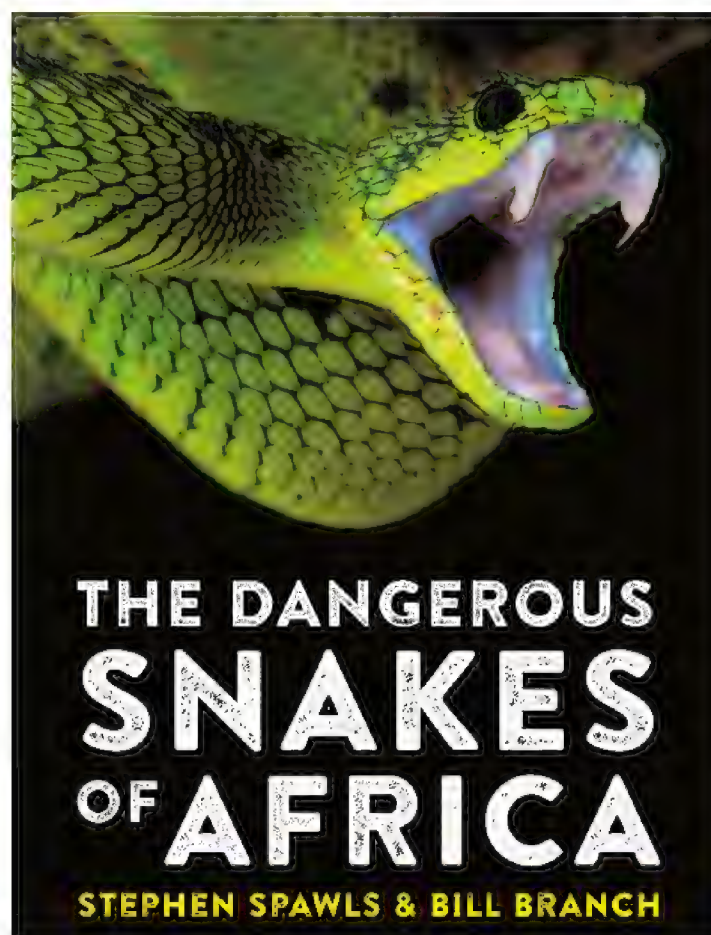


Fig. 1. *The Dangerous Snakes of Africa*. Authors: Stephen Spawls and Bill Branch. Princeton University Press, Princeton, New Jersey, USA. Published 4 August 2020.

Paperback | Price: US \$35.00 / £30.00 | ISBN: 9780691207926
Pages: 336 | Size: 5.25 x 8.5 in. | Illus: 650+ color photographs and maps.

identification may be easier—but make sure the snake is actually dead before handling it, as some dangerous snakes may feign death defensively! For example, turn the snake on its back—if it flips back over, it's not dead. Look for rhythmic waves along the body or if the tail coils and uncoils. If any of these movements are observed, then the snake may be fatally injured but it is not safe to handle. After making sure the snake is dead, various measurements and diagnostics can be taken to aid in identification.

The bulk of the book consists of the species accounts. The accounts are separated into two groups: (1) dangerous front-fanged snakes (130+ species) and (2) dangerous rear-ranged and fangless snakes (17 species). Each account contains four to five sections: Identification, Habitat and Distribution, Natural History, Medical Significance, and sometimes Taxonomic Notes. Each account is accompanied by several color photos of the snake and a range map. The accounts are 1–2 pages long, written without technical jargon, and easy to read. Scattered throughout the accounts are keys to help identify various species within one genus, especially if a particular genus has several species that are difficult to tell apart. Special care was taken to make the Medical Significance section as complete and as up to date as possible. Information covered includes the type of venom (cytotoxic, haemorrhagic, neurotoxic, etc.), if antivenom is needed (or if even available), bite and venom symptoms, bite behavior (frequent “dry” bites, aggressiveness, etc.), and other important information that may help when treating bite victims.

The 17 species covered in the “dangerous rear-ranged and fangless snakes” section fall within a few groups based on the dangers they pose, rather than taxonomic groups. These include the pythons which are powerful enough to kill humans, rear-fanged snakes that have been known to kill people (mainly snake handlers), and fangless snakes whose bites are dangerous due to their toxic saliva. The accounts follow the same format as in the “dangerous front-fanged snakes” section.

Following the two dangerous snake sections is a “look-alikes and common species” section. The 70 or so species featured in this section do not have species accounts, rather, a brief description is provided along with a list of similar-looking dangerous species and a few photos. These short write-ups also include distinctive characteristics that the look-alike species have that the dangerous species may lack (but not always, hence the confusion).

Following the look-alike and common species section is an essay entitled, “Snakebite in Africa: the big picture.” Here, the authors discuss various public health issues related to snakebite, such as pharmaceutical companies and their decisions on whether to make antivenom or not, African healthcare, and the apparent randomness of snake bites. In addition, the authors compare Australia with Africa in regard to snake bites. Australia has more species of venomous snakes than harmless ones, and yet the number of snake bites is relatively small. Most Australians are affluent and medically snake aware. They are able to adequately seal their houses against snakes, wear strong footwear when entering wild areas, and farming practices are mechanized. The situation in Africa is much different—the population is poorer, not as well informed, and measures for protection from snakes are not as great. The authors explore some solutions to address the situation, such as improving living standards, increasing snake bite awareness, creating a network of clinics, and increasing regional cooperation.

Much work still needs to be done on these fronts.

Additional sections in the book include tips for avoiding snakebite (both in the home and outside in the field), who is at risk (when and where), what happens when a snake bites and how bad will it be, an incredibly important section on snakebite first aid (do’s and don’ts), and treatment of snakebite at medical centers. There is also a brief section on eye and face first aid for spitting snakes. The authors go into great detail about antivenom, such as syndromes of envenoming, when antivenom should be used, administration of antivenom, and use of antivenom by a lay person.

The book concludes with several valuable appendices: current producers of snakebite antivenoms useful for Africa; important references, forums, and websites; and a checklist of dangerous snakes from the regions and countries of Africa. A list of medical and snakebite terms, a glossary, and an index are also included.

Although snakes are often feared, and a first response when seeing a snake—dangerous or not—is to kill it, snakes have benefits. Their venoms are pharmaceutically important for drug research and advancements. Snakes benefit humans indirectly as important members of the global fauna and have their place in food webs and landscape ecology. The authors argue that snake threats must be taken in context. A cobra on school grounds may obviously need to be dealt with, but a snake crossing a road in a wildlife refuge can be appreciated without the need to interact. To determine if an African snake is potentially dangerous—use this book! Use your best judgement and don’t unnecessarily handle or otherwise disturb snakes. Snakes tend to avoid humans and make efforts to avoid detection. In conservation terms, snakes are not actually threatened by direct killing or by commercial collection. The biggest threats are habitat destruction and conversion to large-scale farms and logging operations. Many of the African snakes covered in this book occur in small patches of habitat and these remaining refuges are being threatened daily by human encroachment. If Africa’s herpetofauna is to survive, habitat conservation needs to be a mainstream goal and local human populations need to understand that these reserves are beneficial in a practical or aesthetic way. We have a long way to go in achieving a balance between human appreciation of nature and recognizing when herpetofauna actually pose a real threat. *The Dangerous Snakes of Africa* is a first step toward approaching this balance.

Literature Cited

- Clark HO Jr. 2012. Rattlesnake mimicry in the Pacific Gopher Snake (*Pituophis catenifer catenifer*). *Sonoran Herpetologist* 25(8): 78.
- Conradie W, Grieneisen ML, Hassapakis CL (Editors). 2019. Compilation of personal tributes to William Roy Branch (1946–2018): a loving husband and father, a good friend, and a mentor. *Amphibian & Reptile Conservation* 13(2) [Special Section]: i–xxix (e186).



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A new species of *Tantilla* of the *taeniata* group (Squamata: Colubridae) from Refugio de Vida Silvestre Barras de Cuero y Salado in Caribbean coastal Honduras

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Abstract.—A new species of *Tantilla* is described from the Refugio de Vida Silvestre Barras de Cuero y Salado (RVSBCS), on the Caribbean coast of Honduras. Assigned to the *Tantilla taeniata* group, this species differs from others in this group in color pattern, numbers of scales, measurements, and habitat. An incomplete pale nuchal collar and a pale mediodorsal stripe extending to the proximal edge of the paravertebral rows on the anterior third of the body are present. The lateral extension of the head cap does not completely separate the postocular pale spot from the pale nuchal collar. A pale lateral stripe is present on the adjacent halves of dorsal scale rows 3 and 4. The ventrolateral ground color is much darker than that of the dorsolateral ground color. The ventral + subcaudal number of 244 is the highest figure for the males of species in the group. The RVSBCS is an important coastal protected area in Mesoamerica, due to its significant coastal diversity, including iconic species, in addition to harboring this centipede snake.

Keywords. Centipede snake, Departamento de Atlántida, protected area, Reptilia, Río Salado, taxonomy

Resumen.—Describimos una nueva especie de *Tantilla* del Refugio de Vida Silvestre Barras de Cuero y Salado (RVSBCS), en la costa caribeña de Honduras. Asignada al grupo *Tantilla taeniata*, esta especie difiere de otras en este grupo en cuanto a patrón de color, número de escamas, medidas y hábitat. Están presentes un collar nuchal pálido incompleto y una franja mediodorsal pálida que se extiende hasta el borde proximal de las filas paravertebrales en el tercio anterior del cuerpo. La extensión lateral de la tapa de la cabeza no separa completamente la mancha pálida postocular del collar nuchal pálido. Una franja lateral pálida está presente en las mitades adyacentes de las filas de escamas dorsales 3 y 4. El color de fondo ventrolateral es mucho más oscuro que el color de fondo dorsolateral. El número ventral + subcaudal de 244 es la cifra más alta para los machos de las especies del grupo. El RVSBCS es una importante área costera protegida en Mesoamérica, ya que tiene una importante diversidad costera, incluidas especies icónicas, además de albergar a esta serpiente tragaciempiés.

Palabras Claves. Área protegida, Departamento de Atlántida, Reptilia, Río Salado, serpiente ciempiés, taxonomía

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Introduction

The colubrid genus *Tantilla* currently consists of 66 species (Wilson 1982; Wilson and Mata-Silva 2015; Batista et al. 2016; Koch and Venegas 2016; Hofmann et al. 2017; McCranie and Smith 2017; Uetz et al. 2020).

Collectively, members of this genus are distributed from portions of many US states (Virginia, Indiana, Illinois, Missouri, Nebraska, Kansas, Colorado, Utah, Nevada, and California), southward through the peninsula of Baja California, most of mainland Mexico, throughout Central America, and into South America (as far south

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as southern Peru, Bolivia, northern Argentina, and Uruguay). This genus also occurs on Isla del Carmen in the Gulf of California, the Tres Marias Islands off the Pacific coast of mainland Mexico, Isla Cozumel off the coast of the Yucatan Peninsula, the Bay Islands off the northern coast of Honduras, and Trinidad and Tobago in the British West Indies (Wilson 1999: 26). Due in part to their cryptozoic nature, relatively few specimens of many of the 66 described species have been collected, and 13 are known thus far only from their respective holotypes (Wilson and Mata-Silva 2015; Batista et al. 2016; Hofmann et al. 2017; McCranie and Smith 2017).

Wilson (1999) divided the genus *Tantilla* into five phenetic groups: *T. calamarina*, *T. coronata*, *T. melanocephala*, *T. planiceps*, and *T. taeniata*. These groups collectively contained 37 of the 53 species (69.8%) included in the genus at the time that paper was written. As noted by Wilson and Mata-Silva (2015: 451), “Wilson and Mata-Silva (2014) suggested that [*Tantilla rubra*] could be one of three (including *T. bocourti* and *T. cucullata*...) that might comprise a so-called *rubra* group” and that “Dixon et al. (2000) provided partial support for this hypothesis, by indicating that *T. cucullata* presumably is the sister taxon of [*T. rubra*].” Eleven of the 13 species described or resurrected from synonymy subsequent to Wilson (1999) have been allocated to the *calamarina* group (*T. ceboruca*, *T. sertula*), the *melanocephala* group (*T. armillata*, *T. boipiranga*, *T. ruficeps*), or the *taeniata* group (*T. excelsa*, *T. gottei*, *T. hendersoni*, *T. olympia*, *T. psittaca*, *T. stenigrammi*). Two additional species described after Wilson (1999) have not been allocated to a phenetic group, i.e., *T. robusta* (Canseco-Márquez et al. 2002) and *T. tjiasmantoi* (Koch and Venegas 2016); the latter species, however, appears to resemble *T. semicineta* (Wilson 1976), in that both species have a pattern of dark transverse bands. Holm (2008) allocated *T. alticola*, *T. bairdi*, *T. moesta*, *T. schistosa*, and *T. semicineta* to the *taeniata* group and *T. petersi* to the *melanocephala* group. However, that work remains unpublished; therefore, its conclusions have not been subjected to peer review so they are considered as unsubstantiated and not followed here. Holm (2008) also noted that *T. albiceps*, *T. nigra*, *T. shawi*, and *T. supracincta* have many unique character states making them difficult to allocate to a species group; and we agree with this statement for the reasons indicated. This statement also seems applicable to *T. robusta*, although Canseco-Márquez et al. (2002) remarked that this species resembles *T. schistosa* in color pattern. This species also can be noted to resemble *T. alticola* in the same way.

Initially, Wilson and Meyer (1971) divided the *Tantilla taeniata* group into six species, distributed geographically from Oaxaca in Mexico to northwestern Colombia. This is currently the largest group in the genus, including 25 described species (Smith and Williams 1966; Wilson 1983; McCranie 2011b; Townsend et al. 2013; Batista et al. 2016; McCranie and Smith 2017), which comprises

37.9% of the 66 species now recognized (*The Reptile Database*; accessed 13 May 2020). As noted in the recent revision of McCranie and Smith (2017: 338), “the *Tantilla taeniata* group members are characterized by the possession of dark dorsal surfaces with pale middorsal and lateral stripes, and by having a pale nuchal collar. Those stripes are occasionally reduced to dashes or dots in a few species, and the nuchal collar is complete, incomplete, or reduced in a few species.”

In May 2018, a distinctively patterned *Tantilla* was collected from a coastal locality within the boundaries of Refugio de Vida Silvestre Barras de Cuero y Salado (RVSBCS) in Honduras. The specimen exhibits the general characteristics of coloration used to define members of the *Tantilla taeniata* group, but it also exhibits clear diagnostic differences from all nominal species in terms of coloration, features of scutellation, measurements, and habitat. Efforts to collect additional specimens of *Tantilla* from RVSBCS (in September 2018, November 2018, and May 2019) were unsuccessful, but we consider the characteristics of the single specimen to be sufficiently distinctive to warrant recognition as a distinct species, which is described herein.

Materials and Methods

The description of the holotype follows those in Campbell (1998), McCranie (2011b), Townsend et al. (2013), and McCranie and Smith (2017). Morphological measurements were made with an analogue caliper Mitutoyo +0.02 mm series (No. 51490093) and an LW Scientific DM Series Stereoscopic Microscope. A considerable amount of time was spent examining the shapes, sizes, and proportions of the scales of the head following Savage (1973), and determining the numbers of ventral, dorsal, and subcaudal scales following Dowling (1951). The following measurements were recorded: total length (TOL); snout-vent length (SVL), taken from the tip of the rostral to the posterior edge of the cloacal scute; tail length (TAL), taken from the posterior edge of the cloacal scute to the tip of the tail; head length (HL), taken from the tip of the rostral to the posterior end of the upper jaw; and head width (HW), taken at the widest part of the head. The lengths and widths of some head scales were measured to provide a more detailed description of the specimen.

The color pattern of the holotype in life is described based on digital photographs taken with a Canon Rebel T3 Camera, as well as the pattern after the specimen was preserved in alcohol, following Campbell (1998). The letter codes of the colors in parentheses below are based on Köhler (2012). The patterns and types of colors and morphological measurements (including numbers and shapes of the scales), are compared between the specimen collected and all known species of the *Tantilla taeniata* group, based on the data in Townsend et al. (2013), Batista et al. (2016), and McCranie and Smith (2017).



Fig. 1. Dorsolateral view of the holotype of *Tantilla lydia* sp. nov. (UVS-V 1189) in life. Photo by Cristopher Antúnez-Fonseca.

The description of the hemipenis follows the descriptions of *T. psittaca* (McCranie 2011b), *T. olympia* (Townsend et al. 2013), and *T. hendersoni* (Hofmann et al. 2017).

Following the morphological species limits within the *Tantilla taeniata* group by Campbell and Smith (1997), Campbell (1998), and McCranie and Smith (2017), the definition of this new species is based on characteristic features of color pattern, such as the middorsal and lateral stripes; the nuchal collar; the coloration of the head, dorsum, and venter; the numbers of ventral, subcaudal, dorsal, and head scales; and the total length, snout-vent length, and tail length. This new species is described based only on the holotype, following the procedures in Campbell and Smith (1997), Stafford (2004), Townsend et al. (2013), and Batista et al. (2016).

Results

Tantilla lydia sp. nov.

Figs. 1–2.

Suggested common name. Lydia's Little Snake.

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Holotype. An adult male, Universidad Nacional Autónoma de Honduras en Valle de Sula ([UVS-V] 1189), from Comunidad Salado Barra in Refugio de Vida Silvestre Barras de Cuero y Salado (15.7633°N, 86.9948°W), elevation 7 m asl, Municipio de El Porvenir, Departamento de Atlántida, Honduras, collected 21 May 2018 by Cristopher Antúnez-Fonseca, Farlem España, Jocelyn Castro, Emmanuel Orellana, José Paz, and Lourdes Alvarado. Original field number CS 15.

Diagnosis. *Tantilla lydia* sp. nov. is a member of the *Tantilla taeniata* species group, but distinguished from all other congeners by possessing the following combination of characteristics: (1) pale middorsal stripe dark-edged, occupying middorsal scale row and adjacent third of paravertebral rows on anterior third of body, reducing to median half of vertebral row on remainder of body, beginning approximately on tenth middorsal scale past parietals, posterior to more or less circular pale spot just posterior to dark nape band located behind pale nuchal collar; (2) pale nuchal collar incomplete dorsally, divided by dark coloration on vertebral scales and connecting to dark posterior border of dark head cap and dark nape band; (3) lateral extension of dark head cap incomplete, not completely separating postocular pale spot from pale nuchal band; (4) subocular dark spot present, not extending to lip; (5) ventrolateral region of body a much darker shade of brown than dorsolateral region; (6) pale lateral stripe well defined, dark edged, located on adjacent halves of dorsal scales 3 and 4; (7) paraventral scale completely pale on anterior portion, gradually darkening dorsally, until becoming completely dark at the beginning of tail; (8) postnasal and preocular narrowly separated; (9) 169 ventrals, 75 subcaudals, and 244 ventrals + subcaudals in the single male holotype.

Tantilla lydia can be differentiated from the other members of the *T. taeniata* group (Tables 1–2) by having (scutellation data for males only): 169 ventrals (vs. 152 in *T. berguidoi*, 139–152 in *T. brevicauda*, 172 in *T. briggsi*, 139–145 in *T. cuniculator*, 154–166 in *T. flavilineata*, 142–158 in *T. gottei*, 157 in *T. hendersoni*, 162–165 in *T. impensa*, 144–147 in *T. jani*, 144–159 in

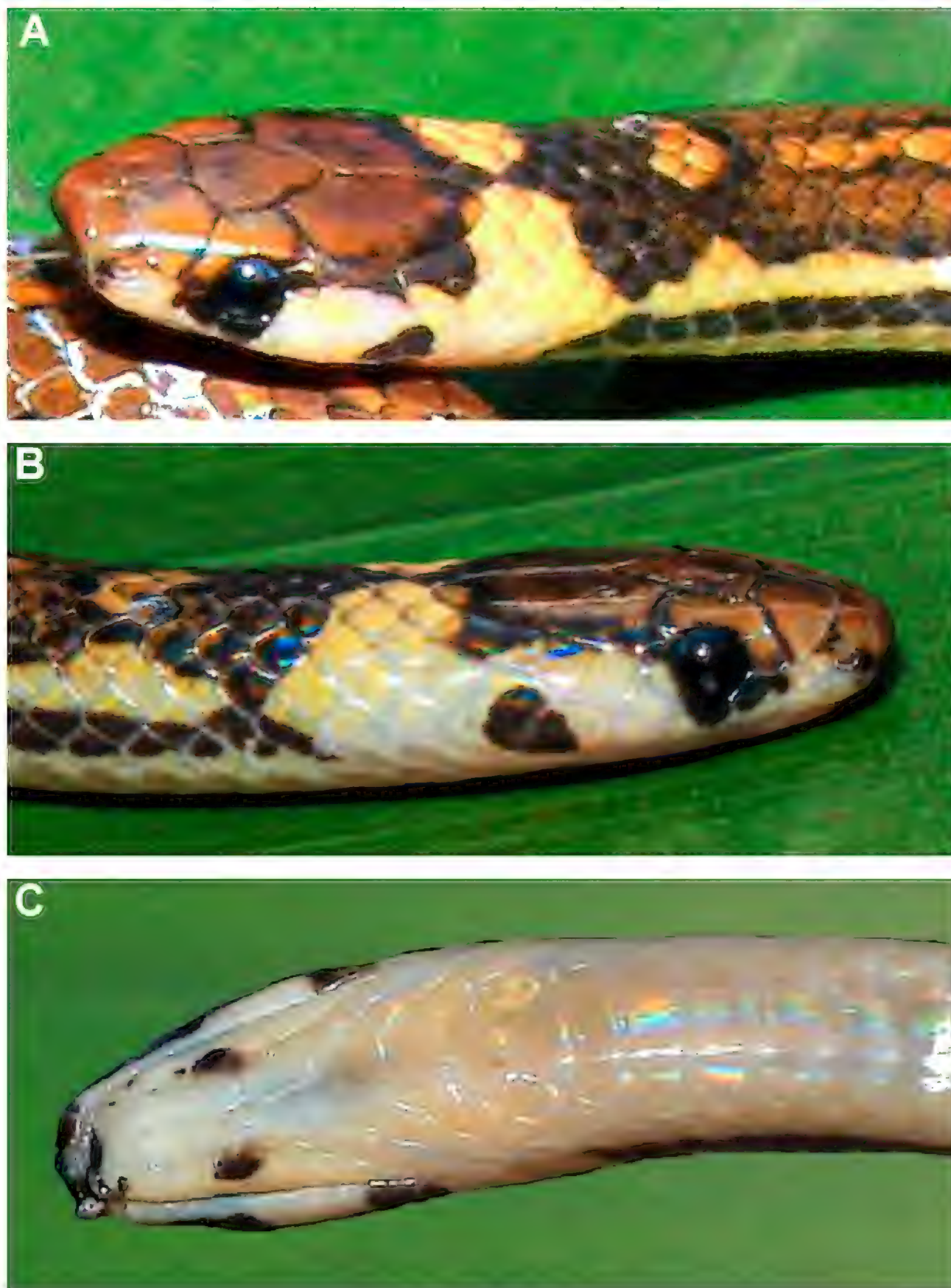


Fig. 2. Dorsal (A), lateral (B), and ventral (C) views of the head and nape of the holotype of *Tantilla lydia* **sp. nov.** (UVS-V 1189). Photos by Cristopher Antúnez-Fonseca.

T. johnsoni, 151–158 in *T. oaxacae*, 148 in *T. olympia*, 153–163 in *T. psittaca*, 158–159 in *T. reticulata*, 164 in *T. stenigrammi*, 146–161 in *T. striata*, 141–152 in *T. taeniata*, 140–144 in *T. tayrae*, 157 in *T. tritaeniata*, and 136–146 in *T. vulcani*); 75 subcaudals (vs. 65 in *T. berguidoi*, 22–26 in *T. brevicauda*, 68 in *T. briggsi*, 53–58 in *T. cuniculator*, 70 in *T. excelsa*, 51–56 in *T. flavilineata*, 62–67 in *T. gottei*, 70 in *T. hendersoni*, 68–72 in *T. impensa*, 44–47 in *T. jani*, 62 in *T. johnsoni*, 46–52 in *T. oaxacae*, 49 in *T. olympia*, 63–73 in *T. psittaca*, 60–67 in *T. reticulata*, 33–42 in *T. striata*, 60–70 in *T. taeniata*, 46–49 in *T. tayrae*, and 39–50 in *T. vulcani*); pale nuchal band narrowly divided middorsally (vs. obscure but complete in *T. berguidoi*, complete dorsally in *T. brevicauda*, *T. cuniculator*, *T. excelsa*, *T. flavilineata*, *T. gottei*, *T. johnsoni*, *T. stenigrammi*, *T. taeniata*, *T. tecta*, *T. trilineata*, and *T. triseriata*, and reduced to two nuchal spots in *T. striata*); by having nuchal band extending

onto parietals (vs. nuchal band confined to scales posterior to parietals in *T. hendersoni*, *T. slavensi*, and *T. tayrae*); pale middorsal stripe occupying middorsal scale row and adjacent portions of paravertebral rows on anterior third of body, narrowing to median portion of middorsal scale row on remainder of body (vs. confined to median portion of middorsal scale row length of body in *T. berguidoi*, restricted to spots on vertebral row in *T. brevicauda*, *T. jani*, *T. olympia*, and *T. vulcani*, absent in *T. briggsi*, *T. cuniculator*, and *T. johnsoni*, absent or barely indicated, consisting of series of disjunct paler spots on anterior portion of middorsal scales length of trunk or some portion of anterior end thereof in *T. tayrae*, present on middorsal scale row and some portion of paravertebral scale rows length of body in *T. excelsa*, *T. flavilineata*, *T. gottei*, *T. oaxacae*, *T. psittaca*, *T. reticulata*, *T. striata*, *T. taeniata*, and *T. tritaeniata*, confined to middorsal scale row length of body in *T. hendersoni*, *T. impensa*,

Table 1. Selected features of measurements, proportion, and scutellation of the members of the *Tantilla taeniata* group. Modified from Townsend et al. (2013).

Species	Maximum total length (mm)	Ventrals (♂)	Subcaudals (♂)	Ventrals (♀)	Subcaudals (♀)	Tail/total length ratio (%)
<i>T. lydia</i> sp. nov.	344	169	75	—	—	23.8
<i>T. berguidoi</i>	408	152	65	—	—	25.2
<i>T. breviceauda</i>	171	139–152	22–26	148–160	21–22	9.9–12.9
<i>T. briggsi</i>	301	172	68	—	—	22.6
<i>T. cuniculator</i>	220	139–145	53–58	140–154	48–53	19.7–22.9
<i>T. excelsa</i>	400	169	70	161–178	61	23.0–24.0
<i>T. flavilineata</i>	293	154–166	51–56	152–168	43–49	17.7–20.6
<i>T. gottei</i>	391	142–158	62–67	147	61–70	23.0–26.0
<i>T. hendersoni</i>	358	157	70	151–153	64	23.9–24.9
<i>T. impensa</i>	ca. 725	162–165	68–72	164–172	65–72	21.0–25.0
<i>T. jani</i>	242	144–147	44–47	144	47	15.7–20.7
<i>T. johnsoni</i>	353+	144–159	62	—	—	22.5
<i>T. oaxacae</i>	284	151–158	46–52	145	45–48	19.9–21.2
<i>T. olympia</i>	338	148	49	—	—	20.7
<i>T. psittaca</i>	413	153–163	63–73	154–161	—	24.1–25.2
<i>T. reticulata</i>	312	158–159	60–67	162–173	59–70	21.7–24.1
<i>T. slavensi</i>	346	—	—	158–159	52–56	19.9–24.6
<i>T. stenigrammi</i>	173+	164	—	159	—	—
<i>T. striata</i>	217	146–161	33–42	145–163	31–34	13.0–17.0
<i>T. taeniata</i>	415	141–152	60–70	150	59	23.0–27.0
<i>T. tayrae</i>	360	140–144	46–49	146–154	44–51	18.5–20.3
<i>T. tecta</i>	222	—	—	148	54	23.0
<i>T. trilineata</i>	Tail incomplete	—	—	149	41+	—
<i>T. triseriata</i>	375	—	—	159–167	58–63	19.7–22.2
<i>T. tritaeniata</i>	273	157	—	155–161	59–65	22.7–23.6
<i>T. vulcani</i>	247	136–146	39–50	141–154	38–47	15.4–22.0

T. tecta, and *T. trilineata*, confined to middorsal scale row, becoming increasingly obscured and fragmented posteriorly in *T. slavensi*, and confined to middorsal scale row anteriorly and extending onto adjacent edges of paravertebral scale rows posteriorly on body in *T. stenigrammi*, *T. tecta*, and *T. triseriata*); pale lateral stripe well-defined, occupying adjacent portions of dorsal scale rows 3 and 4 (vs. occupying dorsal scale 4 and adjacent halves of rows 3 and 5 in *T. berguidoi*, *T. excelsa*, *T. flavilineata*, *T. oaxacae*, *T. reticulata*, and *T. stenigrammi*, poorly defined, occupying all of row 4, upper half of row 3, and sometimes lower portion of row 5 in *T. breviceauda*, interrupted on adjacent portion of scale rows 3 and 4 in *T. briggsi*, barely discernible on adjacent portions of scale rows 3 and 4 in *T. cuniculator*, absent or occupying portion of adjacent portions of scale rows 3 and 4, most clearly or barely evident on anterior portion of trunk in *T. johnsoni* and *T. tayrae*, well-defined, consisting of spots on scale row 4 in *T. olympia*); paraventral scale pale anteriorly, gradually darkening until reaching tail (vs. uniformly tan, brown, or dark brown length of body in *T. berguidoi*, *T. breviceauda*, *T. cuniculator*, *T. jani*, *T. johnsoni*, *T. oaxacae*, *T. reticulata*, *T. striata*, *T. tayrae*, *T. tecta*, and *T. vulcani*, lower portion

pale, distinctly set off from dark upper half length of body in *T. briggsi*, *T. gottei*, *T. hendersoni*, and *T. impensa*, lower two-thirds anteriorly and about lower one-third posteriorly white similar to color of ventrals in *T. excelsa*; dark streak on posterior portion of otherwise pale colored scale in *T. flavilineata*, with pale center, edged with dark pigment in *T. olympia*, lower two-thirds pale, area with pale pigment slightly decreasing posteriorly on body in *T. psittaca*, lower half pale, distinctly set off from dark brown upper half in *T. slavensi* and *T. taeniata*, lower half to two-thirds of scale row 1 colored similarly to ventrals in *T. stenigrammi*, unpigmented on anterior half or more of body, upper half darkly pigmented thereafter in *T. triseriata*, lower tip pale, decreasing in amount of coverage posteriorly in *T. tritaeniata*); and by venter immaculate white (vs. increasingly involved with ventral edge of ventrolateral dark stripe proceeding toward tail tip in *T. berguidoi*, sometimes lightly pigmented in *T. breviceauda*, immaculate cream anteriorly to pale pink posteriorly in *T. briggsi*, immaculate reddish-orange in *T. cuniculator*, white with little or no dark spotting in *T. excelsa*, scattering of brown pigment in *T. flavilineata*, edged with dark brown spotting in *T. jani*, with slight extension of tan coloration of first scale row

Table 2. Selected characteristics of the color pattern in members of the *Tantilla taeniata* group. Modified from Townsend et al. (2013).

Species	Nuchal band	Pale middorsal stripe	Pale lateral stripe	Paraventral scale	Lateral edges of ventral scales
<i>T. lydia</i> sp. nov.	Interrupted dorsally, extends onto parietals, crosses last supralabial	Occupying middorsal scale row and adjacent third of paravertebral rows on anterior third of body, reducing to middorsal row on remainder	Occupies adjacent halves of scale rows 3 and 4	Pale anteriorly, gradually darkening until reaching tail	Immaculate white
<i>T. berguidoi</i>	Obscure, beginning on posterior portions of parietals and extending posteriorly onto middorsal scale immediately behind median parietal suture, laterally grading into pale pigment on posterior portion of last supralabial and nuchal scale posterior to last supralabial and posterior temporal scale	Confined to median portion of middorsal scale row	Occupies dorsal scale 4 and adjacent halves of scale rows 3 and 5	Uniformly dark brown	Increasingly involved with ventral edge of ventrolateral dark stripe proceeding toward tail tip
<i>T. brevicauda</i>	Complete dorsally, extends onto parietals and does or does not cross last supralabial	Reduced to spots on vertebral scales	Poorly defined, occupying upper half of row 3, all of row 4, and sometimes lower portion of row 5	Uniformly brown to dark brown	Sometimes lightly pigmented
<i>T. briggsi</i>	Interrupted dorsally, extends onto parietals and crosses last supralabial	Absent	Interrupted on adjacent halves of scale rows 3 and 4	Lower half pale, distinctly set off from dark upper half	Immaculate cream anteriorly to pale pink (red-orange in life?) posteriorly
<i>T. cuniculator</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Absent	Barely discernible on adjacent halves of scale rows 3 and 4	Uniformly brown	Immaculate reddish-orange
<i>T. excelsa</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Extends along the body at least to middle of tail, on the vertebral row and adjacent third of paravertebral rows	Well defined on adjacent halves of rows 3 and 4	Lower two-thirds anteriorly and about lower third posteriorly of scale row 1 white similar to color of ventrals	White with little or no dark spotting
<i>T. flavilineata</i>	Complete dorsally, does or does not extend onto parietals and crosses last supralabial	Occupies middorsal and adjacent halves of paravertebral scale rows	Well defined on row 4 and adjacent halves of rows 3 and 5	Dark streak on posterior portion of otherwise pale colored scale	Scattering of brown pigment
<i>T. gottei</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Extends length of the body and most of tail on vertebral row and adjacent third of paravertebral rows	Adjacent third to three-quarters of scale rows 3 and 4	Lower two-thirds of row 1 scales colored similarly to ventrals	Immaculate white to yellow

Table 2 (continued). Selected characteristics of the color pattern in members of the *Tantilla taeniata* group. Modified from Townsend et al. (2013).

Species	Nuchal band	Pale middorsal stripe	Pale lateral stripe	Paraventral scale	Lateral edges of ventral scales
<i>T. hendersoni</i>	Interrupted or complete dorsally, does not extend onto parietals, but does cross last supralabial	Confined to middorsal scale row	Occupies adjacent thirds of scale rows 3 and 4	Lower half pale, distinctly set off from dark upper half	Immaculate white
<i>T. impensa</i>	Complete or interrupted dorsally, extends onto parietals or not and crosses last supralabial	Occupies medial two-thirds of middorsal scale row	Occupies adjacent halves of scale rows 3 and 4	Lower half cream to yellow, upper half dark brown	Immaculate cream
<i>T. jani</i>	Interrupted dorsally and laterally, medial portion extends onto parietals and lateral portion crossing last supralabial	Reduced to series of small spots on vertebral scale row	Narrow, occupying adjacent thirds of scale rows 3 and 4	Primarily uniformly dark brown	Edged with dark brown spotting
<i>T. johnsoni</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Absent	Absent or occupying portion of adjacent halves of scale rows 3 and 4, most clearly on anterior portion of trunk	Uniformly tan to dark tan	Immaculate cream
<i>T. oaxacae</i>	Usually interrupted dorsally, extends onto parietals, but does not cross last supralabial	Occupies middorsal and adjacent halves of paravertebral scale rows	Well defined on row 4 and adjacent halves of rows 3 and 5	Uniformly tan	Slight extension of tan coloration of first scale row
<i>T. olympia</i>	Interrupted dorsally, extends onto parietals and crosses last supralabial	Reduced to series of spots on vertebral scale row (one per scale)	Well defined, consisting of spots on 4 th scale row	Pale center, edged with dark pigment	Darkly pigmented
<i>T. psittaca</i>	Complete dorsally or not, extends onto parietals and crosses last supralabial	Occupies middorsal and adjacent one third to one half of paravertebral scale rows	Occupies adjacent halves of scale rows 3 and 4	Lower two thirds pale, area with pale pigment slightly decreasing posteriorly on body	Immaculate pink anteriorly grading to red on posterior two-thirds of body
<i>T. reticulata</i>	Interrupted dorsally, extends onto parietals and crosses last supralabial	Occupies middorsal and adjacent halves of paravertebral scale rows	Well defined on row 4 and adjacent halves of rows 3 and 5	Uniformly pale brown	Darkly pigmented
<i>T. slavensi</i>	Interrupted dorsally, confined to scales posterior to parietals, crosses last supralabial	Confined to middorsal scale row, becoming increasingly obscured and fragmented posteriorly	Occupies adjacent thirds of scale rows 3 and 4	Lower half pale, distinctly set off from dark brown upper half	Immaculate orange

Table 2 (continued). Selected characteristics of the color pattern in members of the *Tantilla taeniata* group. Modified from Townsend et al. (2013).

Species	Nuchal band	Pale middorsal stripe	Pale lateral stripe	Paraventral scale	Lateral edges of ventral scales
<i>T. stenigrammi</i>	Complete dorsally, extends onto the edges of the parietals and crosses the last supralabial	Confined to middorsal scale row, at least on anterior half and extending onto adjacent edges of paravertebral scale rows posteriorly on body	Occupies adjacent halves of rows 3 and 4	Lower half to two-thirds of scale row 1 colored similarly to ventrals	Immaculate white
<i>T. striata</i>	Reduced to two nuchal spots, extending onto parietals or not and crossing last supralabial or not	Occupies middorsal and adjacent halves of paravertebral scale rows	Occupies adjacent halves of scale rows 3 and 4	Uniformly pale brown	Immaculate cream
<i>T. taeniata</i>	Usually complete dorsally, extends onto parietals and crosses last supralabial	Occupies middorsal and adjacent halves of paravertebral scale rows	Occupies adjacent halves of scale rows 3 and 4	Lower half pale, distinctly set off from dark upper half	Usually immaculate cream anteriorly grading to yellow posteriorly, but sometimes with a few small dark spots
<i>T. tayrae</i>	Poorly indicated, interrupted dorsally or dorsally and laterally, confined to scales posterior to parietals, crosses last supralabial	Absent or barely indicated, consisting of series of disjunct slightly paler spots on anterior portion of middorsal scales the length of the trunk or some portion of anterior end thereof	Absent or barest indication of one on adjacent halves of scale rows 3 and 4 on anterior portion of trunk	Uniformly dark brown	Dark spot on extreme anterolateral portion of each ventral
<i>T. tecta</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Confined to middorsal scale length of body	Occupies adjacent halves of scale rows 3 and 4	Uniformly brown	Edged with color same as that of paraventral row
<i>T. trilineata</i>	Complete dorsally, position relative to parietals not documented, crosses last supralabial	Confined to middorsal scale row	Occupies adjacent halves of scale rows 3 and 4	Unknown	Immaculate cream
<i>T. triseriata</i>	Complete dorsally, extends onto parietals and crosses last supralabial	Confined to middorsal scale row anteriorly, expanding to adjacent halves of paravertebral rows posteriorly	Occupies adjacent halves of scale rows 3 and 4	Unpigmented on anterior half or more of trunk, upper half of scale darkly pigmented thereafter	Immaculate pale yellow
<i>T. tritaeniata</i>	Interrupted dorsally, in some cases also laterally, extends onto parietals, and crosses last supralabial	Occupies middorsal and one third to two-thirds of paravertebral rows	Occupies adjacent two-thirds of scale rows 3 and 4	Lower tip pale, decreasing in amount of coverage posteriorly	Immaculate cream
<i>T. vulcani</i>	Usually complete dorsally, extends onto parietals and crosses last supralabial	Reduced to series of small spots on vertebral scale row (one per scale)	Occupies adjacent halves of scale rows 3 and 4	Uniformly brown	Darkly edged with color similar to that of paraventral row; remainder of venter white

in *T. oaxacae*, darkly pigmented in *T. olympia* and *T. reticulata*, immaculate pink anteriorly grading to red on posterior two-thirds of body in *T. psittaca*, immaculate orange in *T. slavensi*, usually immaculate, but sometimes with a few small dark spots in *T. taeniata*, dark spot on extreme anterolateral portion of each ventral in *T. tayrae*, and edged with same color as that of paraventral row in *T. tecta*, and darkly edged with color similar to that of paraventral row, remainder of venter white in *T. vulcani*).

Description of holotype (Figs. 1–2). An adult male, with partially everted hemipenes, measuring 262 mm SVL and 82 mm TL (TOL = 344 mm; 23.9% of TOL). The head is slightly broader than the attenuate body; HL 8.5 mm; HW 5.1 mm; ED 1.4 mm, about 16.5% of HL; snout length 4.8 mm, about 56.4% of HL; snout rounded in dorsal and lateral views; pupil circular; rostral in the shape of an inverted triangle (2.1 mm in length by 1.1 mm in width), 1.9 times wider than long; internasal (0.9 mm in length by 1.7 mm in width), 1.9 times wider than long, contacting anterior and posterior nasal, and relatively large nostril; short suture between pre- and postnasals, below nostril; prefrontal more or less quadrangular (1.7 mm in length by 1.9 mm in width), anterior portion wider than the posterior portion prefrontal, 1.5 times longer than intersuture length; parietal (4.0 mm in length by 2.1 mm in width), about 1.9 times longer than wide; prefrontal suture 1.2 mm in length; frontal (2.5 mm in length by 1.9 mm in width), pentagonal in shape, approximately 1.3 times longer than wide, approximately as long as the distance from its anterior edge to tip of snout; supraocular (1.9 mm in length by 0.9 mm in width) approximately 2.1 times longer than wide; central portion of parietal 1.9 times longer than wide; parietals in contact with five nuchal scales; border of orbit in contact with parietal, upper postocular, supraocular, and frontal; rostral in contact with anterior nasal, internodal, and supralabial 1; anterior nasal in contact with the rostral, nostril, and first supralabial; posterior nasal in contact with nostril, prefrontal, and supralabials 1 and 2; relatively large nasal fossa located between anterior nasal and posterior nasal; loreal absent; preocular single, of inverted pentagonal shape (0.7 x 0.9 mm), lower margin contacting supralabials 2 and 3; postoculars 2/2, upper scale of roughly pentagonal shape (0.8 x 0.6 mm); temporals 1+1, anterior temporal (1.9 x 0.9 mm) longer than wide, posterior temporal (2.0 x 1.0 mm) longer than wide; supralabials 7/7, supralabial 1 in contact with supralabial 2 and nasals, supralabial 2 in contact with supralabial 1 and 3, preocular, and prefrontal, 3 and 4 bordering the eye, 4 and 5 contacting the lower postocular, 5, 6, and 7 bounding the ventral border of the anterior temporal, 7 contacting the anterior and posterior temporal, and the scales of the pale collar; a pair of chin shields present, anterior ones 1.7 times longer than wide, in contact with infralabials 1, 2, 3 and 4, infralabial 6/6, first four in contact with chin shields; and four preventral

scales present between the posterior chin shields and first ventral. Dorsal scales in 15-15-15 smooth rows throughout the body, without apical pits or supra-cloacal tubercles; dorsal scales 6 at the 10th subcaudal; ventral 169; cloacal shield divided; subcaudals 75, paired; ventrals plus subcaudals 244. Hemipenes slightly everted, bilobed, well-differentiated, pedicel naked and smooth, apical region with large spines.

Coloration of holotype in life (Figs. 1–2). Dark dorsolateral region of body Prout's Brown (47); pale middorsal stripe Clay Color (18) present on the middorsal scales and one-fourth of the adjacent portion of the paravertebral scales on anterior portion of body up to ventral 38, thence narrowing to cover only the median two-thirds of middorsal scale on remainder of body, edged with Sepia (286); pale lateral stripe located on adjacent halves of dorsal scale rows 3 and 4 Chamois (84) in color, grading to Tawny Olive (17) on posterior portion of body, bordered on upper half of row 4 with Sepia (286), ventrolateral portion of body from ventral half of row 3 to dorsal portion of row 1 Sepia (286); ventral portion of scale row 1 Smoky White (261); dorsal surface of head from rostral to anterior two-thirds of parietals Buff (15), with Sepia (286) edging on some scale edges; posterior portion of head cap edged with Sepia (286) margin on lateral edges of parietals, upper postocular, upper edge of anterior temporal, upper half of posterior temporal, and anterior half of adjacent nuchal scale; this dark head cap margin confluent with Sepia (286) subocular spot on anterior edge of lower postocular, upper portions of supralabials 3 and 4, and posterodorsal corner of supralabial 2, not touching lip; iris Jet Black (300); lateral portion of head Pale Buff (1), except for Sepia (286) spot on adjacent portions of supralabials 6 and 7, representing isolated segment of lateral extension of head cap, completely separated from dorsal portion of head cap; pale preocular and postocular spots confluent below dark subocular spot; pale nuchal band Light Buff (2) grading to Pale Buff (1) laterally, extending onto posterior tips of parietals where color is Yellow Ocher (14), narrowly divided by middorsal connection between posterior edge of head cap and a Sepia (486) nape band three middorsal scales long, which abuts and edges posteriorly a Yellow Ocher (14) spot covering most of four dorsal scales and is separated from pale middorsal stripe, which begins about one scale posterior to that point; venter of head Pale Buff (1), with Sepia (286) spot on mental and similarly-colored spots on medial portion of each infralabial 4; venter of body and tail Pale Buff (1).

Coloration of holotype in preservative. After seven months of preservation, the holotype exhibited the following coloration: dark dorsolateral region of body Drab (19), located between two pale stripes; Smoky White (261) stripe with Sepia (286) edges covering the vertebral scales and one-fourth of the adjacent portion

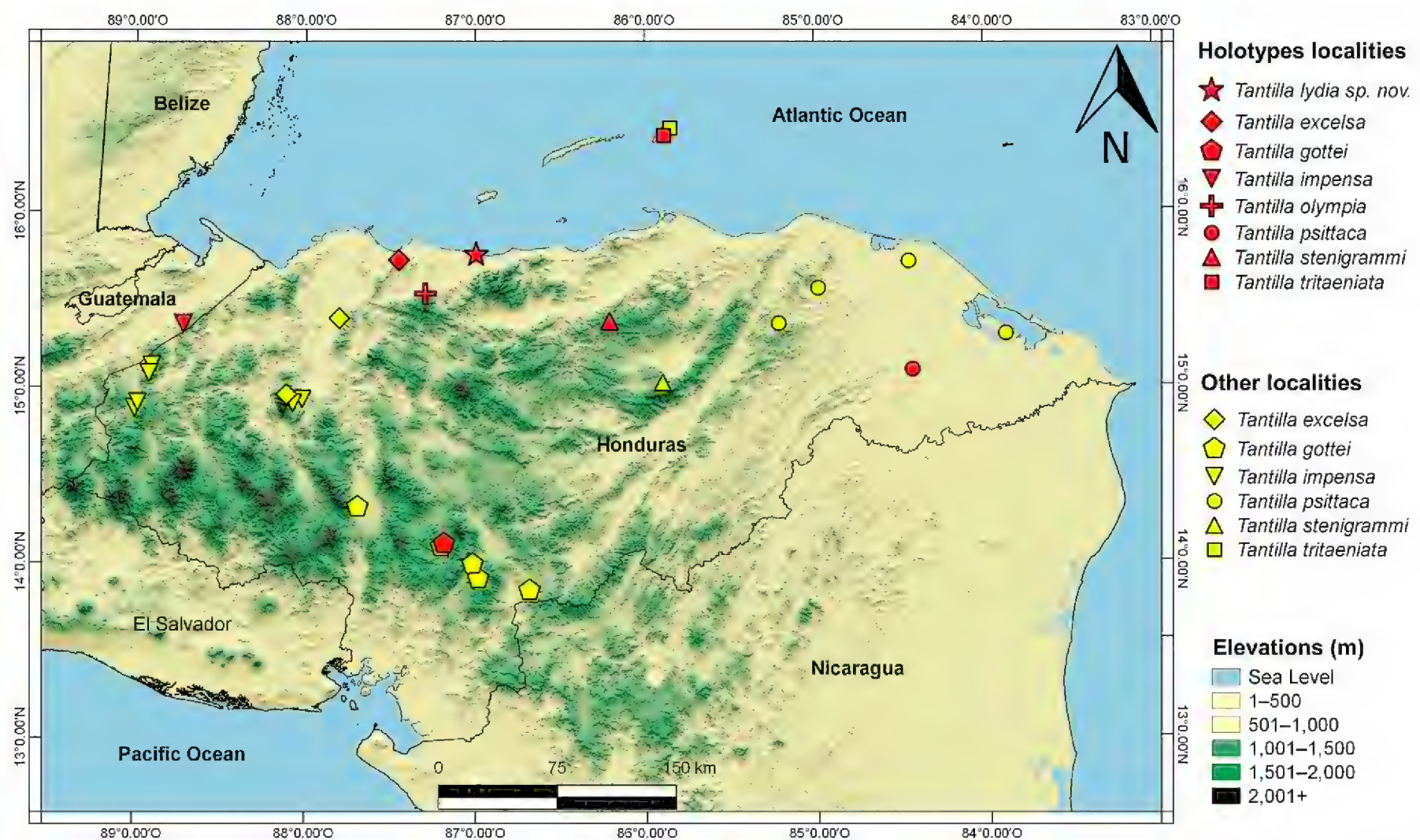


Fig. 3. Distribution of the species of the *Tantilla taeniata* group in Honduras. The star indicates the type locality of *Tantilla lydia* sp. nov. The most northwestern location of *Tantilla gottei* was recently published by Orellana Murillo et al. (2020).

of the paravertebrals, to a point 38 ventral scales along the body, after which this stripe narrows to occupy only middorsal scale row for remainder of body; adjacent portions of dorsal scales 3 and 4 Pale Buff (1), edged by Sepia (286) above, area below lateral pale stripe Hair Brown (276); paraventral portion of dorsal scale row 1 immaculate Pale Buff (1), as are the ventral scales. Dorsal head cap is Hair Brown (276), rimmed on posterior portion by Sepia (286); pale nuchal color is Pale Buff (1), divided narrowly middorsally by a Sepia (286) line connecting posteriorly to the Sepia (286) nape band; side of head is Pale Buff (1), with a Sepia (286) subocular spot not touching lip and a Sepia (286) spot on posterior portion of supralabial six and anterior portion of supralabial seven; chin Pale Buff (1) colored with Sepia (286) spots on mental and fourth infralabials.

Etymology. We are privileged to name this new species of snake in honor of Dr. Lydia Allison Fucsko who resides in Melbourne, Australia, and is an amphibian conservationist and environmental activist. As an internationally published photographer, she has taken countless pictures of amphibians, including photo galleries of mostly southeastern Australian frogs. Dr. Fucsko has a Bachelor of Arts in Humanities from La Trobe University (Bundoora, Victoria, Australia), and a Diploma in Education from The University of Melbourne (Parkville, Victoria, Australia). She has postgraduate diplomas in computer education and in vocational education and training from The University of Melbourne (Parkville). Additionally, Dr. Fucsko holds a Master's Degree in Counseling from Monash University (Clayton, Victoria, Australia). She received

her Ph.D. on environmental education, which promoted habitat conservation, species perpetuation, and global sustainable management, from Swinburne University of Technology (Hawthorn, Victoria, Australia), while being mentored by the late world-renowned Australian herpetologist and academic Dr. Michael James Tyler (Order of Australia recipient). Dr. Fucsko, an educational consultant, was responsible for major enhancements in the quality of the images provided herein and is also a research collaborator with the fifth author (LDW). Dr. Fucsko's academic interests include: clinical psychology, focusing on psychopathology; neuroscience and empathy; environmental education for sustainable development; sentient ecology; academic writing; and creative writing, including poetry and creative nonfiction books for children and young adults. We use Dr. Fucsko's given name as a noun in apposition, with the spelling of the Latin transliteration from the Ancient Greek Λυδία (Ludia), meaning "beauty, beautiful, noble one." Thus, the snake named here as *Tantilla lydia* sp. nov. can be envisioned as the "beautiful one."

Distribution and habitat (Figs. 3–4). *Tantilla lydia* sp. nov. is known only from a narrow strip of disturbed Coastal Scrub habitat in the Lowland Wet Forest (LWF; Holdridge 1967). In the vicinity of the holotype collection location, the predominant plant families and species are: Myrtaceae (*Syzygium cumini*, Indian Blackberry or Malabar Plum); Arecaceae (*Elaeis guianensis* and *Cocos nucifera*, African Oil Palm and Coconut Palm, respectively); Melastomataceae (*Conostegia xalapensis*, Canelito); Fabaceae (*Abrus precatorius*, Rosary Pea);

A new species of *Tantilla* from Honduras

Table 3. Selected features of distribution and conservation status of the members of the *Tantilla taeniata* group. Country distribution abbreviations as follows: Belize = B; Colombia = C; Costa Rica = CR; El Salvador = ES; Guatemala = G; Honduras = H; Mexico = M; Nicaragua = N; Panama = P. Ecological formations are abbreviated as follows: LAF = Lowland Arid Forest, LDF = Lowland Dry Forest, LMF = Lowland Moist Forest, LWF = Lowland Wet Forest, PDF = Premontane Dry Forest, PMF = Premontane Moist Forest, PWF = Premontane Wet Forest, LMDF = Lower Montane Dry Forest, LMMF = Lower Montane Moist Forest, LMWF = Lower Montane West Forest. EVS = Environmental Vulnerability Scores (explained in text). EVS categorization as follows: M = medium; H = high. IUCN categorization as follows: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; LC = Least Concern; DD = Data Deficient; and NE = Not evaluated. Conservation priority levels are explained in the text.

Species	Country distribution	Ecological distribution	Elevational distribution	Versant distribution	EVS	EVS category	IUCN category	Conservation priority level
<i>T. lydia</i> sp. nov.	H	LMF	7 m	Atlantic	16	H	NE	One
<i>T. berguidoi</i>	P	PWF	1,376 m	Pacific	16	H	NE	One
<i>T. brevicauda</i>	G, ES	PMF, PWF, LMWF	1,200–1,510 m	Pacific	13	M	LC	Eight
<i>T. briggsi</i>	M	LMF	95 m	Atlantic	16	H	DD	One
<i>T. cuniculator</i>	B, M	LAF, LDF	near sea level–100 m	Atlantic	13	M	LC	Seven
<i>T. excelsa</i>	H	LDF, LMF, PWF	30–700 m	Atlantic	13	M	NE	Eight
<i>T. flavilineata</i>	M	LMDF, LMMF	1,800–2,300 m	Atlantic	14	H	EN	One
<i>T. gottei</i>	H	LDF, PDF, PMF	500–1,280 m	Pacific	14	H	NE	One
<i>T. hendersoni</i>	B	PMF	194–580 m	Atlantic	16	H	DD	One
<i>T. impensa</i>	G, H, M	LMF, PMF, PWF, LMWF	near sea level–1,600 m	Atlantic	10	M	LC	Eight
<i>T. jani</i>	G	PWF	1,050 m	Pacific	14	H	VU	Two
<i>T. johnsoni</i>	M	LDF	450 m	Pacific	16	H	DD	One
<i>T. oaxacae</i>	M	PMF, LMMF	600–1,600 m	Pacific	15	H	DD	One
<i>T. olympia</i>	H	PWF	1,150 m	Atlantic	16	H	NE	One
<i>T. psittaca</i>	H	LMF	5–420 m	Atlantic	15	H	VU	One
<i>T. reticulata</i>	C, CR, N, P	LMF, PWF	10–1,345 m	Atlantic and Pacific	13	M	LC	Nine
<i>T. slavensi</i>	M	LMF, PWF	50–800 m	Atlantic	14	H	DD	One
<i>T. stenigrammi</i>	H	PMF	895–1,180 m	Atlantic	15	H	NE	One
<i>T. striata</i>	M	LDF, PMF	0–1,500 m	Pacific	14	H	DD	Two
<i>T. taeniata</i>	G	PMF	1,020–1,550 m	Pacific	14	H	LC	Two
<i>T. tayrae</i>	M	LMF, PWF	500–1,000 m	Pacific	15	H	DD	One
<i>T. tecta</i>	G	LDF	220 m	Atlantic	16	H	DD	One
<i>T. trilineata</i>	Unknown	Unknown	Unknown	Unknown	—	—	—	—
<i>T. triseriata</i>	M	LDF, PMF, PWF	500–1,200 m	Atlantic and Pacific	13	M	DD	Eight
<i>T. tritaeniata</i>	H	LWF	near sea level	Caribbean insular	16	H	CR	One
<i>T. vulcani</i>	G, M	LDF, LMF, PWF	500–700 m	Pacific	12	M	NE	Seven

and Marantaceae (*Thalia geniculata*, Fire-flag). The male holotype of this snake was found active on 21 May 2018 during a night with clear skies at 2230 h, between the rails of the old Standard Fruit Company railroad track, 566 m southwest in a straight line from the center of the Comunidad de Salado Barra, approximately 450 m from the Río Salado, 590 m from the community beach, and 5,900 m from the Comunidad de La Unión. An association of mangrove forest species predominates to the west of the type locality on the banks of the aforementioned river, and includes *Rhizophora mangle* (Red Mangrove), *Conocarpus erectus* (Buttonwood), *Avicennia germinans* (Black Mangrove), and *Laguncularia racemosa* (White Mangrove). *Tantilla lydia* sp. nov. shares its microhabitat with other amphibians and reptiles, such as *Dendropsophus microcephalus*, *Scinax staufferi*, *Smilica baudinii*, *Basiliscus vittatus*, *Coniophanes imperialis*, and *Bothrops asper*.

Conservation status. Applying the IUCN Red List criteria (IUCN 2012; IUCN Standards and Petitions Committee 2019) to *Tantilla lydia*, indicates that this species should be considered Critically Endangered (B1ab[iii]) due to the known distribution being limited to a single highly-intervened, threat-defined area of lowland Coastal Strand habitat of < 10 km² in total extent, which has undergone extensive loss of remaining habitat due to deforestation and development. Efforts are underway to restore this habitat, and it is likely that further survey work in nearby coastal areas could uncover additional habitat and/or populations. Given the single known locality of *Tantilla lydia* sp. nov., its unknown population size, unknown extent of geographic and ecological distribution, and significant and continuing degradation of habitat in the vicinity of the type locality, we propose an Environmental Vulnerability Score (EVS) of 16 (6+8+2) within the “High Vulnerability” category (Wilson and McCranie 2003; Johnson et al. 2015).



Fig. 4. Type locality and surrounding habitat for *Tantilla lydia* **sp. nov.** showing the train tracks where the holotype was collected, Comunidad de Salado Barra, La Unión, Departamento de Atlántida, Honduras. Photo by Cristopher Antúnez-Fonseca.

Discussion

This species represents an addition to the genus *Tantilla* (Baird and Girard 1853) and is assigned to the *T. taeniata* group on the basis of features of color pattern. As noted above, the *T. taeniata* group was considered to comprise 25 species until now, with the description of *T. lydia* **sp. nov.** bringing the number to 26. Wilson (1999) listed 18 species for this group: *T. brevicauda*, *T. briggsi*, *T. cuniculator*, *T. flavilineata*, *T. impensa*, *T. jani*, *T. johnsoni*, *T. oaxacae*, *T. reticulata*, *T. slavensi*, *T. striata*, *T. taeniata*, *T. tayrae*, *T. tecta*, *T. trilineata*, *T. triseriata*, *T. tritaeniata*, and *T. vulcani*. Since the summary provided by Wilson (1999), an additional seven species have been described: *T. berguidoi* (Batista et al. 2016), *T. excelsa* (McCranie and Smith 2017), *T. gottei* (McCranie and Smith 2017), *T. hendersoni* (Stafford 2004), *T. olympia* (Townsend et al. 2013), *T. psittaca* (McCranie 2011), and *T. stenigrammi* (McCranie and Smith 2017).

Members of the *T. taeniata* group are distributed in all Mesoamerican countries and the northwestern-most country of South America, i.e., Colombia (Table 3), as follows: Mexico (11 species), Belize (two), Guatemala (six), El Salvador (one), Honduras (eight), Nicaragua (one), Costa Rica (one), Panama (two), and Colombia (one). Most of this group's species are limited in distribution to single countries (i.e., endemic), amounting to 20 of the 26 species (Table 3). Thus, only five of the species are found in more than one country: *T. brevicauda* (Guatemala and El Salvador), *T. cuniculator* (Mexico and Belize), *T. impensa* (Mexico, Guatemala, and Honduras), *T. reticulata* (Nicaragua, Costa Rica, Panama, and Colombia), and *T. vulcani* (Mexico and Guatemala).

Members of the *T. taeniata* group are found in most of the forest formations which occur throughout the group's range (Table 3) at low, moderate, and intermediate elevations (ranging from near sea level to 2,300 m). Seventeen species are distributed at low elevations (sea level to 600 m), sixteen at moderate elevations (601–

1,500 m), and five at intermediate elevations (1,501–2,300 m). More specifically, the numbers of species found in particular forest formations are as follows (Table 3): Lowland Moist Forest (nine species), Lowland Dry Forest (eight), Lowland Arid Forest (one), Premontane Wet Forest (11), Premontane Moist Forest (nine), Lower Montane Wet Forest (two), Lower Montane Moist Forest (two), and Lower Montane Dry Forest (one). Thirteen of the 26 species (50.0%) occupy more than one forest formation; the remainder are found in only a single formation.

Most of the species in the *T. taeniata* group (22 of 26 species; 84.6%) are limited to occurrence on only one versant. Of the 22 single-versant species, 10 are limited to the Pacific versant and 12 to the Atlantic versant. Only two species (*T. reticulata* and *T. triseriata*) occupy both versants, and one other species (*T. tritaeniata*) is of insular distribution (on the Bay Islands of Honduras).

The conservation status of the members of the *T. taeniata* group were examined using the IUCN and EVS systems. The IUCN system is the more broadly used of the two systems, but proves to be less useful for comprehensive conservation assessment than the EVS system (Table 3). For example, the largest number of species (nine) is allocated to the Data Deficient category of IUCN and the next largest (seven) to the Not Evaluated category. These two categories, which divulge no useful information about the conservation status of the species involved, are applied to 16 species, or 61.5% of the 25 species in the *taeniata* group that can be categorized. (Note that *T. trilineata* is too poorly known to allow for categorization, because it is known only from the holotype from an unknown locality). Five species are allocated to the Least Concern category (*T. brevicauda*, *T. cuniculator*, *T. impensa*, *T. reticulata*, and *T. taeniata*). With the exception of *T. taeniata*, the remaining four are the most broadly distributed geographically and ecologically, and are allocated to the threatened categories of Critically Endangered (*T. tritaeniata*), Endangered (*T. flavilineata*), and Vulnerable (*T. jani* and *T. psittaca*).

The EVS system (Wilson et al. 2013a,b; Johnson et al. 2015) is of greater utility, as all species, other than *T. trilineata*, can be categorized (Table 3). The EVS range from 10 to 16, with an average score of 14.4. Eighteen of the 25 species that can be categorized (72.0%) are allocated to the high vulnerability category (with scores ranging from 14 to 16); the remaining seven (28.0%) are placed in the medium vulnerability category (with scores ranging from 10 to 13). Thus, none of the species are allocated to the low category of vulnerability. Typically, Mesoamerican species of *Tantilla* are restricted in distribution and this phenomenon is reflected in their generally high EVS.

Johnson et al. (2017) and Mata-Silva et al. (2019) introduced the concept of conservation priority levels by combining patterns of physiographic distribution with environmental vulnerability scores. These levels

can theoretically range from one to 24 in Mesoamerica, but practically range from one to 18. For the species of the *T. taeniata* group these levels (Table 3) range from one to nine, as follows: one (15 species), two (three), seven (two), eight (four), and nine (one). Fifteen of the 25 species (60.0%) for which the priority levels can be determined are allocated to conservation priority level one and, thus, merit the greatest degree of conservation attention among the species in the *T. taeniata* group (Table 3).

The holotype of *Tantilla lydia* was found in a strip of forest in the “regeneration” stage in the middle of a cultivation of *Cocos nucifera* adjoining a mangrove forest almost 0.5 km from the Río Salado. Although in some respects, the known ecology of *T. lydia* is similar to that of other species within the *T. taeniata* group in Honduras; specifically, all of these species occur in leaf litter, although *T. lydia* occurs at lower elevations than the other species and, unlike the other species in this group, it was found in Lowland Wet Forest (Holdridge 1967). In contrast, *T. excelsa* occurs mainly at higher elevations and almost exclusively in Premontane Moist Forest and Lowland Dry Forest, but also in Lowland Wet Forest and typically in proximity to rivers; *T. gottei* also occurs at higher elevations than *T. lydia* and is found in pine forests within the Premontane Moist Forest and Lowland Dry Forest zones in the middle basin of the Choluteca River in south-central Honduras; *T. impensa* occurs in Tropical and Subtropical Humid Forests, mainly in primary forests and is known to use rotting logs for refuge, as well as leaf litter; *T. olympia* is known from Premontane Moist Forest; *T. psittaca* occurs at similar elevation but in Broadleaf Primary Rain Forest and Pine Savanna, and also occurs in rotting logs; *T. stenigrammi* occurs at higher elevations in disturbed pine-oak forest and Lower Montane Wet Forest adjacent to the Sico Tinto River; and *T. tritaeniata* occurs at similar low elevations as *T. lydia*, but only on Isla Guanaja (Ariano-Sánchez and Sunyer 2013; Campbell 1998; McCranie 2011a; McCranie and Smith 2017; Smith and Williams 1966; Townsend et al. 2013). Thus, *T. lydia* is distinct from other species within the *Tantilla taeniata* group in Honduras in terms of its distribution and ecology, as well as its morphology.

This discovery highlights the fauna of the Refugio de Vida Silvestre Barras de Cuero y Salado (RVSBCS), and the importance of establishing and maintaining a network of protected areas to ensure the conservation of representative communities throughout the country of Honduras. Human activities in the landscape surrounding the RVSBCS involve the maintenance of agricultural systems (i.e., banana, coconut, and African oil palm), livestock production, and human settlements. These activities have reduced significantly the area of the ecosystem within which *T. lydia* evolved. Additionally, existing patches of potential habitat are threatened by continued intensification of these human activities, which results in further reduction in available habitat or

fragmentation that interrupts the connectivity of existing forest patches (Ferrán 1992). As such, the long-term conservation of *T. lydia* is likely at risk. While no attempts are underway to quantify the species’ population status, it is likely to be decreasing, as is the case with many of the other species of flora and fauna restricted to this region.

One final note regarding the taxonomy of the *T. taeniata* group needs to be mentioned. As indicated by McCranie and Smith (2017: 346), “problems remain with the taxonomy of the El Salvadoran and Nicaraguan specimens identified in the literature as *T. taeniata*. Köhler (2003, 2008) and Sunyer and Köhler (2007) provided photographs of recently collected Nicaraguan specimens, and Köhler et al. (2005) included a photograph of a recently collected El Salvador specimen. These specimens also need to be addressed in light of the new taxonomic change[s].” This work remains to be completed.

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Revised Key to Members of *Tantilla taeniata* Group

Townsend et al. (2013) published the most recent key to the members of the *Tantilla taeniata* group. Since that paper appeared, several new species have been described and placed in this group (Batista et al. 2016; McCranie et al. 2017; and herein), so this is an opportune time to revise the key for the identification of 25 of the 26 species now recognized (the information available on *T. trilineata* remains insufficient to include this poorly known taxon in the key; see Tables 1 and 2).

1. Pale middorsal stripe absent.....	2
Pale middorsal stripe present, variously developed.....	6
2. Pale lateral stripe present along length of body.....	3
Pale lateral stripe interrupted along middle of body, present only on anterior portion of body, or absent...4	
3. Pale nuchal band divided middorsally and laterally, pale lateral stripe well developed... <i>T. jani</i> (in part)	
Pale nuchal band complete; pale lateral stripe barely discernible.....	<i>T. cuniculator</i>
4. Pale lateral stripe present but interrupted along middle of body.....	<i>T. briggsi</i>
Pale lateral stripe, if present, confined to anterior portion of body.....	5
5. Pale nuchal band poorly developed, confined to scale posterior to parietals; subcaudals fewer than 60 (known range, 44–51).....	<i>T. tayrae</i> (in part)
Pale nuchal band well developed, extending onto parietals; subcaudals more than 60 (single known value, 62).....	<i>T. johnsoni</i>
6. Subcaudals fewer than 30 (known range, 21–26).....	<i>T. brevicauda</i>
Subcaudals more than 30.....	7
7. Pale lateral stripe occupies rows 4 and adjacent halves of rows 3 and 5.....	8
Pale lateral stripe occupies adjacent halves of rows 3 and 4 or restricted to row 4.....	11
8. Pale nuchal collar does not cross last supralabial.....	<i>T. oaxacae</i>
Pale nuchal collar crosses last supralabial.....	9
9. Pale nuchal collar divided medially; well-developed dark stripe present on lateral edges of ventrals.....	<i>T. reticulata</i>
Pale nuchal collar complete; venter essentially immaculate.....	10
10. Pale middorsal stripe confined to middorsal scale row; subcaudals 65 (single known value).....	<i>T. berguidoi</i>
Pale middorsal stripe occupies middorsal scale row and adjacent halves of paravertebral rows; subcaudals 56 or fewer (known range, 43–56).....	<i>T. flavilineata</i>
11. Pale nuchal band reduced to two nuchal spots.....	<i>T. striata</i>
Pale nuchal band complete, divided medially, or divided both medially and laterally.....	12
12. Pale middorsal stripe on middorsal scale row and some portion of paravertebral rows at least on posterior portion of body.....	13
Pale middorsal stripe confined to middorsal scale row, or on middorsal scale row and some portion of paravertebral rows on anterior portion of body, continuing on or reducing to middorsal row on posterior portion of body.....	19
13. Pale middorsal stripe confined to middorsal scale row anteriorly, expanding to some portion of paravertebral rows posteriorly.....	14
Pale middorsal stripe on middorsal scale row and some portion of paravertebral rows along length of body.....	15
14. Lower half to two-thirds of paraventral scale row colored similarly to ventrals.....	<i>T. stenigrammi</i>
Paraventral scale row pigmented on anterior half or more of trunk, upper half of scale darkly pigmented thereafter.....	<i>T. triseriata</i>
15. Ventral surface some shade of red.....	16
Ventral surface yellow or white.....	17
16. Ventral scales 153 or more (range 153–163).....	<i>T. psittaca</i>
Ventral scales 152 or fewer (range 141–152).....	<i>T. taeniata</i>
17. Pale nuchal collar divided.....	<i>T. tritaeniata</i>
Pale nuchal collar complete.....	18
18. Ventrals 142–158 in both sexes combined.....	<i>T. gottei</i>
Ventrals 161–178 in both sexes combined.....	<i>T. excelsa</i>
19. Pale middorsal stripe on middorsal scale row and adjacent one-third of paravertebral rows on anterior portion of body, reducing to middorsal row on posterior portion of body.....	<i>T. lydia</i>
Pale middorsal stripe confined to middorsal scale row, either as continuous stripe or as fragmented series of spots.....	20

20. Pale middorsal stripe fragmented, consisting of series of isolated spots.....	21
Pale middorsal stripe complete, but confined to middorsal row.....	24
21. Pale lateral stripe consisting of series of spots on dorsal scale row 4.....	<i>T. olympia</i>
Pale lateral stripe absent or present on some portion of dorsal scale rows 3 and 4.....	22
22. Pale lateral stripe absent or barely evident on adjacent halves of dorsal scale rows 3 and 4 on anterior portion of body.....	<i>T. tayrae</i> (in part)
Pale lateral stripe present on adjacent portions of dorsal scale rows 3 and 4 length of body.....	23
23. Pale nuchal band interrupted both dorsally and laterally.....	<i>T. jani</i>
Pale nuchal band usually complete.....	<i>T. vulcani</i>
24. Paraventral scale uniformly brown.....	<i>T. tecta</i>
Paraventral scale divided into dark upper half and pale lower half.....	25
25. Subcaudal scales 56 or fewer (range 52–56).....	<i>T. slavensi</i>
Subcaudal scales 64 or more (combined range 64–72).....	26
26. Ventral scales 157 or fewer (range 153–157).....	<i>T. hendersoni</i>
Ventral scales 162 or more (range 162–172).....	<i>T. impensa</i>

Literature Cited

- Ariano-Sánchez D, Sunyer J. 2013. *Tantilla taeniata*. La Lista Roja de Especies Amenazadas de la UICN 2013: e.T203329A2764084.
- Baird SF, Girard C. 1853. *Catalogue of North American Reptiles in the Museum of the Smithsonian Institution. Part I. Serpentes*. Smithsonian Institution, Washington, DC, USA. 172 p.
- Batista A, Mebert K, Lotzkat S, Wilson LD. 2016. A new species of centipede snake of the genus *Tantilla* (Squamata: Colubridae) from an isolated premontane forest in eastern Panama. *Mesoamerican Herpetology* 3: 949–960.
- Campbell JA. 1998. Comments on the identities of certain *Tantilla* (Squamata: Colubridae) from Guatemala, with the description of two new species. *Scientific Papers, Natural History Museum, University of Kansas* 7: 1–14.
- Campbell JA, Smith EN. 1997. A new species of *Tantilla* (Serpentes: Colubridae) from northeastern Guatemala. *Proceedings of the Biological Society of Washington* 110: 332–337.
- Canseco-Márquez L, Mendelson III JR, Gutiérrez-Mayén G. 2002. A new species of large *Tantilla* (Squamata: Colubridae) from the Sierra Madre Oriental of Puebla, Mexico. *Herpetologica* 58: 492–497.
- Dixon JR, Vaughan RK, Wilson LD. 2000. The taxonomy of *Tantilla rubra* and allied taxa (Serpentes: Colubridae). *Southwestern Naturalist* 45: 141–153.
- Dowling HG. 1951. A proposed system for counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Ferrán FI. 1992. *Los Restos de la Opulencia: Estudio Socio-ambiental del Refugio de Vida Silvestre Barras de Cuero y Salado*. Centro Agronómico Tropical de Investigación Enseñanza (CATIE), Programa Manejo Integrado de Recursos Naturales, Proyecto Rearm/Cuencas, Turrialba, Costa Rica. 89 p.
- Hofmann EP, Gray RJ, Wilson LD, Townsend JH. 2017. Discovery of the first male specimen of *Tantilla hendersoni* Stafford, 2004 (Squamata: Colubridae), from a new locality in central Belize. *Herpetology Notes* 10: 53–57.
- Holdridge LR. 1967. *Life Zone Ecology*. 2nd edition. Tropical Science Center, San José, Costa Rica. 206 p.
- Holm PA. 2008. Phylogenetic biology of the burrowing snake tribe Sonorini (Colubridae). Ph.D. Dissertation, University of Arizona, Tucson, Arizona, USA. 242 p.
- IUCN. 2012. *IUCN Red List Categories and Criteria: Version 3.1*. 2nd edition. IUCN, Gland, Switzerland and Cambridge, United Kingdom. 32 p.
- IUCN Standards and Petitions Committee. 2019. Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. Available: <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [Accessed: 23 February 2020].
- Johnson JD, Mata-Silva V, Wilson LD. 2015. A conservation reassessment of the Central American herpetofauna based on the EVS measure. *Amphibian & Reptile Conservation* 9(2) [General Section]: 1–94 (e100).
- Johnson JD, Wilson LD, Mata-Silva V, García-Padilla E, DeSantis DL. 2017. The endemic herpetofauna of Mexico: organisms of global significance in severe peril. *Mesoamerican Herpetology* 4: 544–620.
- Koch C, Venegas PJ. 2016. A large and unusually colored new snake species of the genus *Tantilla* (Squamata; Colubridae) from the Peruvian Andes. *PeerJ* 4: e2767.
- Köhler G. 2003. *Reptiles of Central America*. Herpeton-Verlag Elke Köhler, Offenbach, Germany. 367 p.
- Köhler G. 2008. *Reptiles of Central America*. 2nd edition. Herpeton-Verlag Elke Köhler, Offenbach, Germany. 400 p.
- Köhler G. 2012. *Color Catalogue for Field Biologists*. Herpeton-Verlag Elke Köhler, Offenbach, Germany. 49 p.
- Köhler G, Vesley M, Greenbaum E. 2005 [dated 2006]. *The Amphibians and Reptiles of El Salvador*. Krieger, Malabar, Florida, USA. 238 p.
- Mata-Silva V, DeSantis DL, García-Padilla E, Johnson

- JD, Wilson LD. 2019. The endemic herpetofauna of Central America: a casualty of anthropocentrism. *Amphibian & Reptile Conservation* 13(1) [General Section]: 1–64 (e168).
- McCranie JR. 2011a. *The Snakes of Honduras: Systematics, Distribution, and Conservation*. Contributions to Herpetology, Volume 19. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 714 p.
- McCranie JR. 2011b. A new species of *Tantilla* of the *taeniata* species group (Reptilia, Squamata, Colubridae, Colubrinae) from northeastern Honduras. *Zootaxa* 3037: 37–44.
- McCranie JR, Smith EN. 2017. A review of the *Tantilla taeniata* species group (Reptilia: Squamata: Colubridae: Colubrinae) in Honduras, with the description of three new species. *Herpetologica* 73: 338–348.
- Orellana Murillo E, Antúnez-Fonseca CA, Townsend JH. 2020. Geographic distribution. *Tantilla gottei* (Gotte's Centipede Snake). *Herpetological Review* 51: 549.
- Savage JM. 1973. A revised terminology for plates in the loreal region of snakes. *British Journal of Herpetology* 5: 360–362.
- Smith HM, Williams KL. 1966. A new snake (*Tantilla*) from Las Islas de la Bahia, Honduras. *The Southwestern Naturalist* 11: 483–487.
- Stafford PJ. 2004. A new species of *Tantilla* (Serpentes: Colubridae) of the *taeniata* group from southern Belize. *Journal of Herpetology* 38: 43–52.
- Sunyer J, Köhler G. 2007. New country and departmental records of herpetofauna in Nicaragua. *Salamandra* 43: 57–62.
- Townsend JH, Wilson LD, Medina-Flores M, Herrera-B LA. 2013. A new species of centipede snake in the *Tantilla taeniata* group (Squamata: Colubridae) from premontane rainforest in Refugio De Vida Silvestre Texiguat, Honduras. *Journal of Herpetology* 47: 191–200.
- Uetz P, Freed P, Hošek J. 2020. *Tantilla*. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 13 May 2020].
- Wilson LD. 1976. Variation in the South American colubrid snake *Tantilla semicineta* (Duméril, Bibron, and Duméril), with comments on pattern dimorphism. *Bulletin of the Southern California Academy of Sciences* 75: 42–48.
- Wilson LD. 1982. A review of the colubrid snakes of the genus *Tantilla* of Central America. *Milwaukee Public Museum Contributions in Biology and Geology* 52: 1–77.
- Wilson LD. 1983. A new species of *Tantilla* of the *taeniata* group from Chiapas, Mexico. *Journal of Herpetology* 17: 54–59.
- Wilson LD. 1985. *Tantilla reticulata*. *Catalogue of American Amphibians and Reptiles* 370.1. 1 p.
- Wilson LD. 1999. Checklist and key to the species of the genus *Tantilla* (Serpentes: Colubridae), with some distributional commentary. *Smithsonian Herpetological Information Service* 122: 1–34.
- Wilson LD, McCranie JR. 2003. The conservation status of the herpetofauna of Honduras. *Amphibian & Reptile Conservation* 3(1): 6–33 (e12).
- Wilson LD, Johnson JD, Mata-Silva V. 2013a. A conservation reassessment of the amphibians of Mexico based on the EVS measure. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 97–127 (e69).
- Wilson LD, Mata-Silva V, Johnson JD. 2013b. A conservation reassessment of the reptiles of Mexico based on the EVS measure. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 1–47.
- Wilson LD, Mata-Silva V. 2014. Snakes of the genus *Tantilla* (Squamata: Colubridae) in Mexico: taxonomy, distribution, and conservation. *Mesoamerican Herpetology* 1: 5–95.
- Wilson LD, Mata-Silva V. 2015. A checklist to the snakes of the *Tantilla* clade (Squamata: Colubridae), with comments on taxonomy, distribution, and conservation. *Mesoamerican Herpetology* 2: 418–498.
- Wilson LD, Meyer JR. 1971. A revision of the *taeniata* group of the colubrid snake genus *Tantilla*. *Herpetologica* 27: 11–40.



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A new species of *Tantilla* from Honduras



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A new species of the genus *Ceratophora* Gray, 1835 (Reptilia: Agamidae) from a lowland rainforest in Sri Lanka, with insights on rostral appendage evolution in Sri Lankan agamid lizards

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Abstract.—The genus *Ceratophora* (horn-lizards) comprises six species, all of which are endemic to Sri Lanka. Herein, a new species of *Ceratophora* is described based on morphological and molecular evidence. The new species is restricted to the Salgala Forest (~300 m asl elevation) in the Kegalle District of Sri Lanka, which is in the northern part of the wet bioclimatic zone. The new species most closely resembles *Ceratophora aspera* Günther, 1864, but can be distinguished from it by body proportions, number of paravertebral and ventral scales, and ND2 mtDNA data. Complete morphological description of two syntypes of *C. aspera* are also provided, in addition to a key to the species of genus *Ceratophora*. The phylogenetic relationships and evolution of rostral appendages in Sri Lankan agamid lizards are discussed in light of new data. According to IUCN Red List criteria, the new species is categorized as Critically Endangered due to its range-restricted habitat. The major threats for this species are habitat loss due to expansion of commercial-scale agriculture and monoculture plantations, as well as illicit forest encroachments.

Keywords. *Cophotis*, *Lyriocephalus*, mtDNA, ND2, syntype, systematics, taxonomy

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Introduction

Sri Lanka and the Western Ghats of India are collectively recognized as a biodiversity hotspot, rich in both diversity and endemism among the herpetofaunal assemblages (Bossuyt et al. 2004; Gunawardene et al. 2007). However, this area supports the highest human population density among the world's biodiversity hotspots (Cincotta et al. 2000; Helgen and Groves 2005). The evolutionary and phylogenetic uniqueness of Sri Lanka's herpetofauna has been well established (Bossuyt et al. 2004, 2005). Despite its small land area, Sri Lanka is geographically diverse as evidenced by the three peneplains of lowland (< 300 m asl), midland (300–900 m asl), and highland (> 900 m asl), that result in an elevation gradient (Cooray 1967). This

geographic variation, in conjunction with variability in annual average precipitation, has resulted in three major bioclimatic zones in Sri Lanka: the dry zone (< 1,000 mm), the wet zone (> 2,500 mm), and the intermediate zone (> 1,500 mm) [Greller and Balasubramaniam 1980]. Further contributing to Sri Lanka's geoclimatic diversity, three distinct mountain ranges of the Central Highland, Rakwana Hills, and the Knuckles Massif, also occur in Sri Lanka (Gunatilleke and Gunatilleke 1990; MoE-SL 2012). These geo-climatic variations have generated an array of environmental gradients, creating niche filters that promote speciation, which has led to the great diversity of the herpetofauna in Sri Lanka. The great richness of Sri Lankan herpetofauna can be attributed to insular radiation, reproductive isolation, and high island-wide

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habitat heterogeneity and environmental complexity (de Silva 2006; Meegaskumbura et al. 2019). Owing to these drivers of diversity, there is a growing interest in herpetological research in Sri Lanka, particularly in taxonomy, phylogenetics, and ecology (Meegaskumbura et al. 2002, 2019; Pyron et al. 2013; Grismer et al. 2016; Karunarathna et al. 2019).

Among Sri Lanka's herpetofaunal assemblages, the agamid lizards warrant scientific attention. Agamids are widespread in the Old World, particularly throughout the Paleotropics, Palearctic, and Australasia. The 21 species of agamid lizards in Sri Lanka belong to six genera, and include 19 (90.5%) species endemic to the island (Somaweera and Somaweera 2009; de Silva and Ukuwela 2020). The agamid genus *Ceratophora* Gray, 1835 is endemic to Sri Lanka and currently comprises five species (Pethiyagoda and Manamendra-Arachchi 1998) with patchy distributions in the tropical montane and lowland humid forests: *C. aspera* Günther, 1864 (CITES Appendix II, and EN), *C. erdeleni* Pethiyagoda and Manamendra-Arachchi, 1998 (CR), *C. karu* Pethiyagoda and Manamendra-Arachchi, 1998 (CITES Appendix I, and CR), *C. stoddartii* Gray, 1834 (CITES Appendix II, and EN), and *C. tennentii* Günther, 1861 (CITES Appendix II, and EN) [Gibson et al. 2020]. *Ceratophora* is a genus of special interest to evolutionary biologists since three of the five species (*C. aspera*, *C. stoddartii*, and *C. tennentii*) possess a prominent rostral appendage, which is absent in the other two (*C. karu* and *C. erdeleni*) [Pethiyagoda and Manamendra-Arachchi 1998].

Rostral appendages (RA) represent tantalizing organs which have evolved independently in a small number of species across a wide range of taxa, and are rarely observed in lizards (Johnston et al. 2012). They have been recorded in the members of the families Dactyloidae and Chamaeleonidae, and in three genera of the agamid subfamily Draconinae (Williams 1979; Macey et al. 2000a; Schulte et al. 2002). RA morphology is profoundly different among different species of *Ceratophora* suggesting the possibility of independent evolution (Johnston et al. 2012). An earlier molecular phylogeny for the group resulted in three equally parsimonious hypotheses for RA evolution in this genus (Macey et al. 2000b; Schulte et al. 2002), suggesting either (1) independent evolution of RA in three lineages of *Ceratophora*; (2) independent evolution of RA in *C. aspera* and in the common ancestor of *C. stoddartii* and *C. tennentii*, and *C. erdeleni*, with subsequent reduction in *C. erdeleni*; or (3) evolution of RA in the common ancestor of all *Ceratophora*, with subsequent independent loss in *C. karu* and *C. erdeleni* (Johnston et al. 2012; Whiting et al. 2015). Further morphological and allometric analyses suggested that RA likely evolved rapidly and independently in the three lineages of *Ceratophora* as a result of sexual selection (in *C. aspera* and *C. stoddartii*) or as a result of natural selection for crypsis (in *C. tennentii*) [Johnston et al. 2012].

Thus far, *C. aspera* has been considered to be the most widely distributed species of the genus, occurring in lowland rainforests and a few submontane forests in the south-western part of Sri Lanka (Bahir and Surasinghe 2005). This species was described from two syntypes (BMNH 1946.8.30.51–52) by Günther (1864) with the locality stated as “Ceylon” (historical name of Sri Lanka) without a precise location. A closer examination of the *C. aspera* type specimens, along with morphological comparisons with additional museum materials and live specimens from several localities in the wet zone of Sri Lanka, revealed differences in morphological and morphometric characters between the northern and southern populations of *C. aspera*. Concordant molecular divergence in the *ND2* mitochondrial DNA gene suggested that the population in the Salgala Forest (northern part of the wet bioclimatic zone; Fig. 1) likely represents a distinct species of *Ceratophora*, which is described herein.

Materials and Methods

Field sampling and specimens. Field surveys were conducted across different locations in Sri Lanka covering several bioclimatic areas (e.g., dry zone, intermediate zone, and wet zone). At each location, the agamid species found were documented. On average, 10 man-hours were spent per location for the survey. During these surveys, behavioral and other aspects of natural history of the focal species were recorded. The ambient temperature and the substrate temperature were measured using a standard thermometer and a N19 Q1370 infrared thermometer (Dick Smith Electronics, Shanghai, China), respectively. The relative humidity and light intensity were measured with a QM 1594 multifunction environment meter (Digitek Instruments Co., Ltd., Hong Kong, China). An eTrex® 10 GPS (Garmin) was used to record elevation and georeference specimen locations. Sex was determined by the presence in males (M) or absence in females (F) of hemipenal bulges and the rostral horn. The conservation status of the species was evaluated using the 2001 IUCN Red List Categories and Criteria Version 3.1 (IUCN Standards and Petitions Subcommittee 2016).

Museum acronyms follow Uetz et al. (2019). The type material discussed in this paper is deposited in the National Museum of Sri Lanka (NMSL), Colombo. Specimens were caught by hand and photographed in life. They were euthanized using halothane and fixed in 10% formaldehyde for two days, washed in water, and transferred to 70% ethanol for long-term storage. Tail tip muscle tissues were sampled before fixation and subsequently stored in 95% ethanol. For comparison, we examined 33 specimens of *Ceratophora*, representing all recognized species of the genus and including type specimens, 12 additional specimens of two *Cophotis* species, and four specimens of *Lyriocephalus*. Specimens

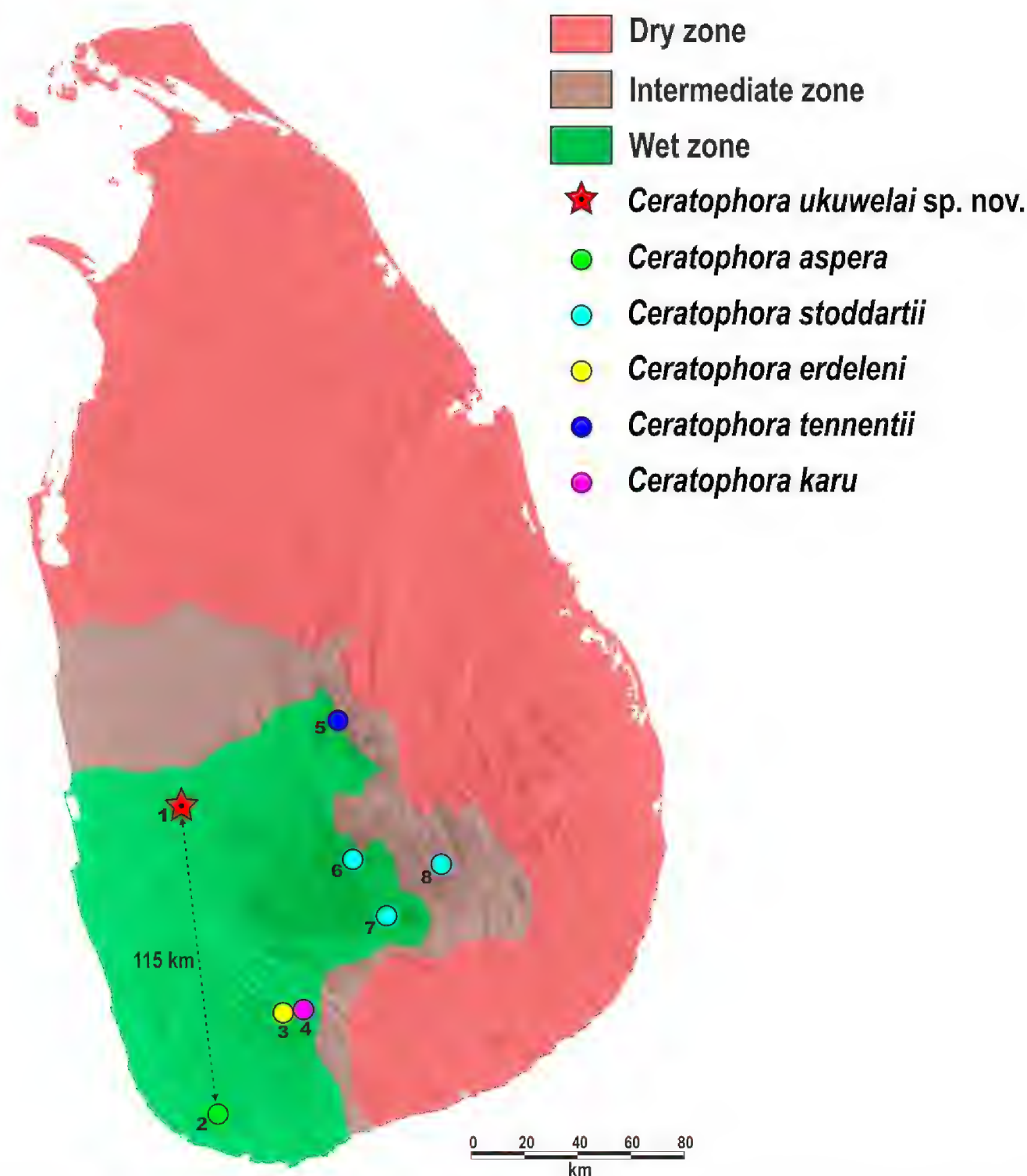


Fig. 1. Currently known distribution of *Ceratophora ukuwelai* sp. nov. and other localities for *Ceratophora* species examined in the present study. For locality numbers see Table 1. Colors of icons correspond to those in Fig. 2. Star denotes the type locality of the new species (Salgala Forest, Kegalle District, Sri Lanka).

that formerly belonged to the Wildlife Heritage Trust (WHT) collection and bearing WHT numbers are currently deposited in the NMSL, catalogued under their original numbers. The full list of comparative materials examined in this study is given in **Appendix 1**. Specimens in this study were collected during a survey of the lizards of Sri Lanka under permit numbers WL/3/2/42/18 (a, 2018 and b, 2019) issued by the Department of Wildlife Conservation, and permit numbers R&E/RES/NFSRCM/Extended/2019, and R&E/RES/NFSRCM/2019-04 issued by the Forest Department of Sri Lanka.

Morphometric characters. Thirty morphometric measurements were taken using a Mitutoyo digital Vernier calliper (to the nearest 0.1 mm), and detailed observations of scales and other structures were made through Leica Wild M3Z and Leica EZ4 dissecting microscopes. The following symmetrical morphometric characters were taken on the left side of the body: RAL, rostral appendage length (distance between tip of snout and tip of horn);

DLM, digit length manus (fork to digit tip, excluding the claw); DLP, digit length pes (fork to digit tip, excluding the claw); EN, eye to nostril length (distance between anteriormost point of bony orbit and middle of nostril); ES, snout length (distance between anteriormost point of bony orbit and tip of snout, excluding appendage); FEL, femur length (distance between groin and knee); HD, head depth (maximum height of head, across eyes); HEL, heel length (from wrist to tip of fourth finger); HL, head length (distance between posterior edge of mandible and tip of snout); HW, head width (maximum width of head); IO, interorbital width (narrowest width across frontal bone); JL, jaw length (from tip of snout to end of mouth corner); LAL, forearm length (distance from elbow to wrist with both upper arm and palm flexed at 90°); OD, orbital diameter (greatest diameter of orbit); PAL, palm length (from ankle to tip of fourth toe); SA, snout to axilla (distance between axilla and tip of snout); SN, snout to nostril (distance between tip of snout and middle of nostril); SVL, snout-vent length (distance between tip of snout and anterior margin of vent); TAL,

tail length (distance between anterior margin of vent and tail tip); TBL, tibia length (distance between knee and heel, with both tibia and tarsus flexed at 90°); TRL, trunk length (distance between axilla and groin); UAL, upper-arm length (distance between axilla and angle of elbow at 90°).

Meristic characters. Twenty discrete characters were recorded using Leica Wild M3Z and Leica EZ4 dissecting microscopes on both the left (L) and the right (R) sides of the body (reported in the form L/R): number of canthal scales (CAS), number of scales from posteriormost point of naris to anterior most point of the orbit; number of enlarged scales on the flanks (FLSP), in between axilla and groin; number of supralabials (SUP) and infralabials (INF) between the first labial scale and the corner of the mouth; number of interorbital scales (INOS), between the left and right supraciliary scale rows; number of midbody scales (MBS) from the center of mid-dorsal row diagonally towards the ventral scales to mid-dorsal; number of midventral scales (MVS) from the first scale posterior to the mental to last scale anterior to the vent; number of dorsal paravertebral scales (PS) between pelvic and pectoral limb insertion points along a straight line immediately left of the vertebral column; number of postmentals (PM) bounded by chin scales, 1st infralabial on the left and right and the mental; number of supraciliary scales (SUS) above the eye; total lamellae on manus I–V (SLM) counted from first proximal enlarged scensor greater than twice width of the largest palm scale to distalmost lamella at tip of digits; total lamellae on pes I–V (SLP), counted from first proximal enlarged scensor greater than twice the width of the largest heel scale to distalmost lamella at tip of digits.

DNA isolation, PCR, and sequencing. To determine the genetic distinctiveness of the new species and its phylogenetic position, a 1,065 bp fragment of *ND2* mitochondrial DNA (mtDNA) gene and adjacent tRNAs were amplified. The *ND2* gene has been widely applied in biodiversity surveys and phylogenetic studies on Sri Lankan agamids, including members of the genus *Ceratophora* (e.g., Macey et al. 2000a; Schulte et al. 2002; Grismer et al. 2016 and references therein). Total genomic DNA was extracted from ethanol-preserved femoral muscle tissue using standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation (protocols followed Hillis et al. 1996). The concentration of total DNA was measured in 1 µl using NanoDrop 2000 (Thermo Scientific, USA), and consequently adjusted to ca. 100 ng DNA/µl.

Polymerase Chain Reaction (PCR) amplifications were performed in 20 µl reactions using ca. 50 ng genomic DNA, 10 nM of each primer, 15 nM of each dNTP, 50 nM additional MgCl₂, Taq PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl₂, and 0.01% gelatin) and 1 U of Taq DNA polymerase. The primers used in PCR

and sequencing included two forward primers: Metf1 (5'-AAGCTTTCGGGCCCATAACC-3'; Macey et al. 1997) and ND2f17 (5'-TGACAAAAAATTGCNCC-3'; Macey et al. 2000b), and two reverse primers: CO1R1 (5'-AGRG TGCCAATGTCTTTGTGRTT-3'; Macey et al. 1997) and ND2r102 (5'-CAGCCTAGGTGGGCGATTG-3'; Greenbaum et al. 2007). The PCR conditions followed Agarwal et al. (2017). PCR products were loaded onto 1% agarose gels and visualized in agarose electrophoresis in the presence of ethidium bromide. PCR products were purified using 2 µl of a 1:4 dilution of ExoSAP-IT (Amersham, United Kingdom) per 5 µl of PCR product prior to cycle sequencing. Purified PCR products were sequenced bidirectionally at Genetech Sri Lanka Pvt. Ltd., Colombo, Sri Lanka. The obtained sequences were deposited in GenBank under the accession numbers MT992241–MT992242 (Table 1).

Phylogenetic analyses. The *ND2* sequences of all *Ceratophora* species and the representatives of all other Draconinae genera for which the homologous sequences were available from GenBank, with the addition of the newly obtained sequences, were used to examine the genealogical relationships within the genus *Ceratophora* (summarized in Table 1). In total, *ND2* sequence data were analyzed for 29 specimens of Draconinae, including nine samples of *Ceratophora*, and the sequence of *Mantheyus phuwuanensis* (Manthey and Nabhitabhata, 1991) was used to root the tree according to its phylogenetic position as the sister lineage to all remaining Draconinae (Grismer et al. 2016). Nucleotide sequences were aligned in MAFFT v.6 (Kato et al. 2002) with default parameters, and subsequently checked visually in BioEdit v.7.0.5.2 (Hall 1999) and slightly adjusted. Mean uncorrected genetic distances (*p*-distances) were calculated in MEGA v.6.0 (Tamura et al. 2013).

The matrilineal genealogy of Draconinae was inferred using Bayesian inference (BI) and Maximum Likelihood (ML) approaches. The optimal evolutionary models for the data set analysis were estimated in MODELTEST v.3.6 (Posada and Crandall 1998). The best-fitting model of DNA evolution for both BI and ML analyses was the HKY+G model for all three codon partitions of the *ND2* gene, as suggested by the Akaike Information Criterion (AIC). BI analysis was conducted in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were performed with one cold chain and three heated chains for 20 million generations and sampled every 2,000 generations. Five independent MCMCMC iterations were performed and the first 1,000 trees were discarded as burn-in. The convergence of the iterations was diagnosed by examining the likelihood plots in TRACER v.1.6 (Rambaut et al. 2014), and the effective sample sizes (ESS) were all above 200. Confidence in nodal topology was estimated by calculating posterior probabilities (BI

Table 1. ND2 mtDNA gene sequences and voucher specimens of Agamidae taxa included in the phylogenetic analyses in this study. For sampling localities of *Ceratophora* species in Sri Lanka see Fig. 1. Sequences generated in this study are marked with an asterisk (*); n-dash (–) denotes no data available.

No.	Species	Locality	Museum ID	GenBank A.N.
1	<i>Ceratophora stoddartii</i>	Sri Lanka: Sita Eliya (6)	WHT1682	AF364053
2	<i>Ceratophora stoddartii</i>	Sri Lanka: Namunukula (8)	WHT1511	AF364054
3	<i>Ceratophora stoddartii</i>	Sri Lanka: Tangamalai (7)	WHT1512	AF128492
4	<i>Ceratophora erdeleni</i>	Sri Lanka: Rakwana (3)	WHT 1808	AF128522
5	<i>Ceratophora tennentii</i>	Sri Lanka: Knuckles (5)	WHT 1633	AF128521
6	<i>Ceratophora karu</i>	Sri Lanka: Rakwana (4)	WHT 2259	AF128520
7	<i>Ceratophora aspera</i>	Sri Lanka: Kottawa (2)	WHT1825	AF128491
8	<i>Ceratophora ukuwelai</i> sp. nov.*	Sri Lanka: Kegalle District: Salgala (1)	NMSL 2020.05.01	MT992241
9	<i>Ceratophora ukuwelai</i> sp. nov.*	Sri Lanka: Kegalle District: Salgala (1)	NMSL 2020.05.02	MT992242
10	<i>Cophotis ceylanica</i>	Sri Lanka: Nagra Division	WHT2061	AF128493
11	<i>Cophotis dumbara</i>	Sri Lanka: Knuckles	–	GQ502785
12	<i>Lyriocephalus scutatus</i>	Sri Lanka: Puwakpitiya	WHT2196	AF128494
13	<i>Aphaniotis fusca</i>	Malaysia: Selangor: Ulu Gombak	TNHC57943	AF128497
14	<i>Harpesaurus borneensis</i>	Malaysia: Sarawak: Kubah N.P.	KUHE 59801	LC469915
15	<i>Cristidorsa otai</i>	Myanmar: Kachin	CAS234833	MK001401
16	<i>Salea kakhienensis</i>	China: Baoshan: Qushi	CAS207492	GQ502784
17	<i>Pseudocalotes brevipes</i>	Vietnam: Vinh Phuc: Tam Dao	MVZ224106	AF128502
18	<i>Diploderma splendida</i>	China: Sichuan: Ya'an	CAS194476	AF128501
19	<i>Acanthosaura lepidogaster</i>	Vietnam: Vinh Phuc: Tam Dao	MVZ224090	AF128499
20	<i>Bronchocela cristatella</i>	Malaysia: Selangor: Ulu Gombak	TNHC57874	AF128495
21	<i>Gonocephalus grandis</i>	Malaysia: Selangor: Ulu Gombak	TNHC56500	AF128496
22	<i>Malayodracon robinsonii</i>	Malaysia: Pahang: Cameron Highlands	LSUHC5873	MK001399
23	<i>Calotes versicolor</i>	Myanmar: Yangon	CAS208157	DQ289478
24	<i>Draco indochinensis</i>	Vietnam: Gia Lai: Ankhe	MVZ222156	AF128477
25	<i>Ptyctolaemus gularis</i>	Myanmar: Kachin: Putao	CAS221515	AY555838
26	<i>Japalura variegata</i>	India: Sikkim: Gangtok	ZISP20922	AF128479
27	<i>Otocryptis wiegmanni</i>	Sri Lanka: Yodaganawa	WHT2262	AF128480
28	<i>Sitana ponticeriana</i>	Sri Lanka: Hambantota	WHT2060	AF128481
29	<i>Mantheyus phuwanensis</i>	Laos: Bolikhamxay: Thaphabat	FMNH255495	AY555836

PP). ML analysis was conducted using the RAxML web server (<https://raxml-ng.vital-it.ch/>; Kozlov et al. 2018). Nodal support was assessed by non-parametric bootstrapping (ML BS) with 1,000 pseudoreplicates (Felsenstein 1985). The nodes with BI PP values > 0.95 and LM BS values $\geq 75\%$ were *a priori* regarded as strongly supported; while BI PP values between 0.95–0.90 and ML BS values between 75–50% were regarded as tendencies. Lower values were regarded as indicating not significantly supported (Huelsenbeck and Hillis 1993).

Divergence time estimations. Molecular divergence dating was performed in BEAST v.1.8.4 (Drummond et al. 2012). An uncorrelated lognormal relaxed clock was set for our data. Substitution models and partitioning

schemes consistently remained the same as those used in the BI and ML phylogeny reconstructions. The Yule model was set as the tree prior and a constant population size and default priors were assumed for all other parameters. Two runs were conducted of 40 million generations, sampling every 4,000 generations, to obtain 10,000 trees for the analysis. We also assumed parameter convergence in Tracer v.1.6 and discarded the first 10% of generations as burn-in. Since no *Ceratophora* fossils are known, we relied on three calibration priors for the subfamily Draconinae obtained from a recent large, phylogenomically-wide revision of agamids (Grismer et al. 2016). Calibration points were as follows: (1) the most recent common ancestor (tMRCA) of the genus *Ceratophora* (18.3 ± 1.8 million years ago [Ma]); (2) tMRCA of the genera *Ceratophora*, *Lyriocephalus*, and

Cophotis (28.1 ± 2.8 Ma); and (3) tMRCA of the genera *Ceratophora*, *Lyriocephalus*, *Cophotis*, *Bronhocela*, *Gonocephalus*, *Aphaniotis*, and *Harpesaurus* (50.8 ± 5.1 Ma).

Rostral appendage evolution analysis. BEAST v.1.8.4 was used to generate a trimmed ultrametric chronogram for the Sri Lankan Draconinae with one specimen per species included in the analysis to investigate the evolutionary history of RA in *Ceratophora* and the closely related genera *Lyriocephalus* and *Cophotis*. The full dataset consisted of all six species of *Ceratophora*, including the newly discovered population of *Ceratophora* sp. from Salgala Forest, two species of *Cophotis*, and a single species of *Lyriocephalus*. Data were added for the newly discovered population of *Ceratophora* sp. from Salgala Forest to the Johnston et al. (2012) morphometric dataset, including data on maximum snout-vent length (SVL), jaw length (JL), head depth (HD), rostral appendage length (RAL), maximal rostral appendage depth (RAD), and relative rostral appendage length (RAL/SVL) and depth (RAD/SVL). The characters were recorded separately for males and females, and measurements followed Johnston et al. (2012). For each species, data were recorded for lifestyle (arboreal or terrestrial), the presence of green colors in the body coloration (yes or no), sexual dimorphism in SVL (yes or no), sexual dimorphism in coloration (yes or no), and sexual dimorphism in rostral appendage morphology (yes or no) [see Table 2]. For *Ceratophora* sp. from Salgala Forest, since no male specimens were collected, measurements were taken in life from a single male, which was subsequently released.

Phylogenetic signal is the tendency for related species to resemble each other more than they resemble species drawn at random from the tree (Blomberg and Garland 2002). The analysis of phylogenetic signal and ancestral state reconstructions were performed in R v.3.6.1 (R Core Team 2020). The phylogenetic signal in phenotypic traits was estimated with Pagel's λ (Pagel 1999) using the 'phylosig' function from the package 'phytools' (Revell 2012). Among several existing tests of phylogenetic signal, Pagel's λ was chosen because it is one of the most reliable tests, and it is robust to the errors in tree topology and branch lengths (Münkemüller et al. 2012; Molina-Venegas and Rodríguez 2017). Pagel's λ was estimated in residual errors of phylogenetic regressions (PGLS) following Revell (2010). PGLS regressions were fit using function 'pgls' from the package 'caper' (Orme et al. 2018). The ancestral state reconstruction was performed using the 'contMap' function from the package 'phytools' (Revell 2013). The aforementioned analyses were performed in R (R Core Team 2020) using RStudio integrated development environment (RStudio Team 2018).

Results

Sequences and statistics. The final alignment of the ND2 gene sequences contained 1,032 aligned characters. Of these, 265 sites were conserved and 767 sites were variable, and 675 of the latter were found to be parsimony-informative. The transition–transversion bias (R) was estimated as 2.16. Nucleotide frequencies (all data given for ingroup only) were 34.91% (A), 23.11% (T), 30.83% (C), and 11.15% (G).

MtDNA genealogy. Both BI and ML analyses resulted in essentially similar topologies, with genealogical relationships varying only in two poorly supported nodes (corresponding to the phylogenetic position of *Cristidorsa otai* (Mahony 2009), and to the position of the clade including genera *Otocryptis* and *Sitana*); all other nodes in the tree were well-resolved and strongly supported (Fig. 2). The BI genealogy inferred the following set of phylogenetic relationships. All Draconinae genera, with the exception of *Mantheyus*, formed two reciprocally monophyletic groups. One of them included the genera *Draco*, *Ptyctolaemus*, and *Japalura* (1.0/97; hereafter node support values are given for BI PP/ML BS, respectively), and another encompassed all the remaining genera of Draconinae (1.0/84). Within the latter clade, the Sri Lankan Draconinae genera were grouped in two subclades: *Otocryptis* + *Sitana* (1.0/100), and the group including *Ceratophora*, *Lyriocephalus*, and *Cophotis* (1.0/99), with the two latter genera forming a well-supported clade (1.0/88); the genus *Cophotis*, including two species *C. ceylanica* and *C. dumbara*, was recovered as monophyletic (1.0/100) [Fig. 2]. Monophyly of the genus *Ceratophora* was strongly supported (1.0/99) and the genealogical relationships within it were well-resolved and strongly supported. Species of *Ceratophora* were grouped into two major reciprocally monophyletic clades: *C. aspera* + *Ceratophora* sp. from Salgala Forest (1.0/100), and the clade joining all the remaining species (1.0/88). Within the latter clade, *C. karu* occupied the most basal position, *C. stoddarti* (1.0/99) was recovered as a sister species of *C. erdeleni* with shallow differentiation between them (1.0/100); while *C. tennentii* formed the sister lineage to (*C. stoddarti* + *C. erdeleni*) (1.0/99) [Fig. 2].

Sequence divergence. The interspecific uncorrected *p*-distances for the ND2 gene fragment within the genus *Ceratophora* varied from *p* = 3.8% (between *C. stoddarti* and *C. erdeleni*) to *p* = 23.0% (between *C. karu* and *Ceratophora* sp. from Salgala Forest) [Table 3]. The newly discovered lineage of *Ceratophora* sp. from Salgala Forest was highly divergent from other congeners and differed from its sister species *C. aspera* by *p* = 9.6% of substitutions in the ND2 gene. This value significantly exceeded the minimal interspecific divergence between *Ceratophora* species (3.8%), as well as the distance

Table 2. Morphological characteristics of adult male (m) and female (f) specimens of nine Sri Lankan agamid species of the genera *Ceratophora* (data partially from Johnston et al. 2012), *Cophotis*, and *Lyriocephalus*. For character abbreviations see **Materials and Methods**; mean values are given for measurements; N: number of specimens, y: yes, n: no.

Species	Lifestyle	Green color	Sexual dimorphism in SVL	Sexual dimorphism in color	Sexual dimorphism in RA	Sex	N	SVL	JL	HD	RAL	RAD	RAL/SVL	RAD/SVL
<i>Cophotis ceylanica</i>	arboreal	y	y	y	n	f	15	66.2	10.4	10.7	0	0	0.00%	0.00%
<i>Cophotis dumbara</i>	arboreal	y	y	y	n	m	5	59.2	13.7	12.2	0	0	0.00%	0.00%
<i>Lyriocephalus scutatus</i>	arboreal	y	y	y	y	m	57	145.6	30.6	45.6	12.4	11.2	8.52%	7.69%
<i>Ceratophora aspera</i>	terrestrial	n	y	n	y	f	26	32.1	8.4	5	0.7	0.5	2.18%	1.56%
<i>Ceratophora ukawelai</i> sp. nov.	terrestrial	n	y	n	y	m	1	36.4	7.4	5.4	4.1	1.5	11.26%	4.12%
<i>Ceratophora karu</i>	terrestrial	n	y	n	y	f	9	27.4	7.8	4.6	0	0	0.00%	0.00%
<i>Ceratophora tennenitii</i>	arboreal	y	n	y	y	m	16	64.5	18.8	11.2	5.6	3.4	8.68%	5.27%
<i>Ceratophora erdeleni</i>	arboreal	y	n	y	n	f	1	55.1	17.2	11	0	0	0.00%	0.00%
<i>Ceratophora stoddartii</i>	arboreal	y	n	y	y	m	40	71.4	22.2	12.8	7	1.7	9.80%	2.38%

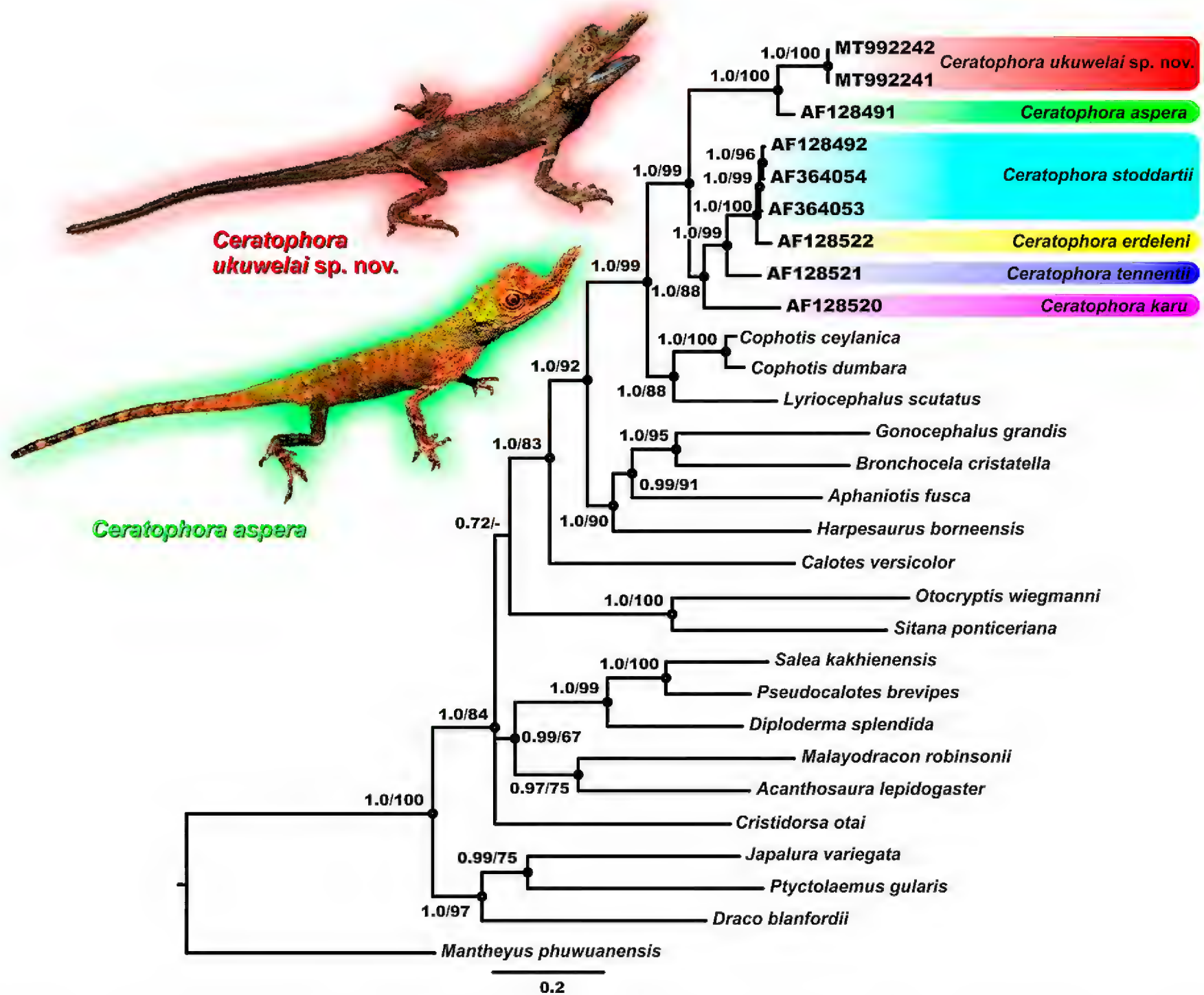


Fig. 2. Bayesian inference tree of Draconinae lizards derived from the analysis of 1,084 bp of *ND2* gene sequences. For voucher specimen information and GenBank accession numbers see Table 1. Numbers at tree nodes correspond to BI PP/ML BS support values, respectively; a black circle at a node indicates it is strongly supported (BI PP > 0.95; ML BS > 75%). Colors of clades and locality numbers correspond to those in Fig. 1. Photos by Sanoj Wijayasekara and Sanjaya Kanishka.

between the two species of *Cophotis* (4.7%) [Table 3]. Deep divergence of the newly discovered *Ceratophora* sp. from Salgala Forest from its congeners indicates that the taxonomy of this group is inconsistent with its phylogeny.

Divergence time estimations. The resulting BEAST chronogram (see Fig. 3) had topology slightly different from the BI tree. Specifically, *Cristidorsa otai* was grouped with the clade including *Salea*, *Pseudocalotes*, *Diploderma*, and *Malayodracon*; *Calotes versicolor* clustered with the *Otocryptis* + *Sitana* clade. These topological differences refer to poorly supported nodes and do not affect the analysis of relationships among Sri Lankan agamids. The time tree analysis (see Fig. 3) reveals that tMRCA of the Sri Lankan agamid clade including *Ceratophora*, *Lyriocephalus*, and *Cophotis* originated during the middle Eocene, ca. 44.14 Ma (36.8–51.19), and radiated within a relatively narrow time period in the middle Oligocene ca. 27.37 Ma (23.3–31.34), which is concordant with the earlier estimate of

Grismer et al. (2016) of 28.1 Ma. A basal split within the genus *Ceratophora* is estimated to have taken place in the early Miocene ca. 19.94 Ma (17.06–22.77), which is slightly earlier than the previous estimate by Schulte et al. (2002), who suggested that the radiation of *Ceratophora* took place in mid-Miocene (13 Ma). The divergence between the ancestors of *C. aspera* and the newly discovered *Ceratophora* sp. from Salgala Forest likely happened in late Miocene, ca. 7.8 Ma (5.43–10.54).

Rostral appendage evolution analysis. In both sexes, the Pagel's λ was close to unity and differed significantly from zero ($p < 0.05$) in most of the characteristics of body size (SVL, JL, HD, and all their \log_{10} -transformed values), indicating a strong phylogenetic signal. The only exception was jaw length in females, for which the Pagel's λ was insignificant ($p = 0.1$). The Pagel's λ values in all measures of the rostrum (length, maximum depth, \log_{10} -transformed length and depth, length and depth relative to SVL, and residuals from the regression

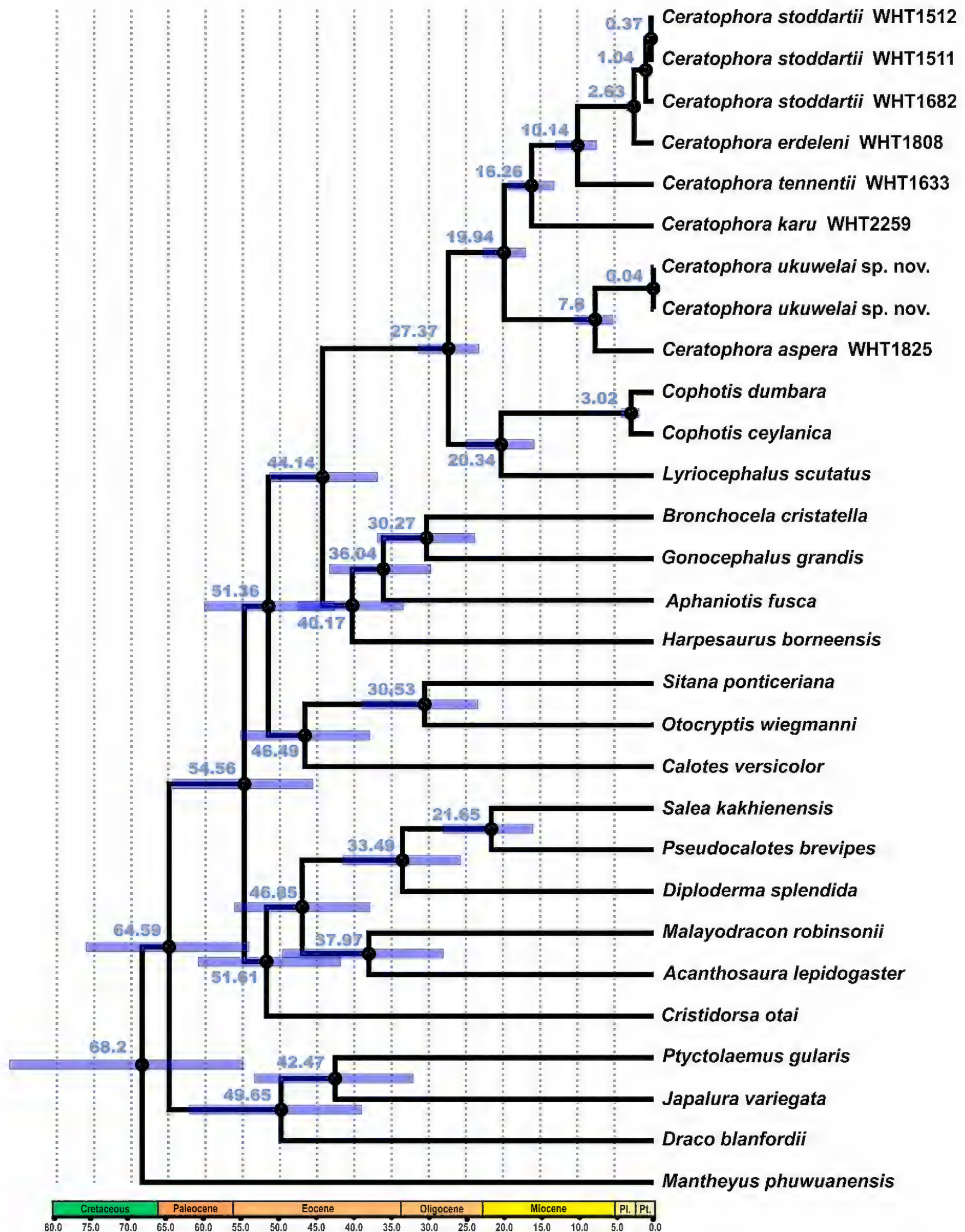


Fig. 3. Bayesian chronogram for Draconinae resulting from BEAST analysis of 1,084 bp of *ND2* gene sequences. Node values correspond to estimated divergence times (in Ma). Blue bars correspond to 95% confidence intervals.

of rostrum length on SVL) were not significantly different from zero ($p = 1$), indicating an absence of phylogenetic signal (see Table 4). The reconstruction of ancestral states for relative rostrum length suggests the presence of RA in tMRCA of *Ceratophora*, *Cophotis*, and *Lyriocephalus* for both sexes and contrasting patterns of RA evolution in males and females (Fig. 4).

Systematics

The results of the updated *ND2*-based genealogy of Sri Lankan agamids are largely consistent with the earlier phylogenies of Schulte et al. (2002), Grismer et al. (2016), Wang et al. (2019), and Kurita et al. (2020). This analysis suggests that the population of *Ceratophora* sp. from

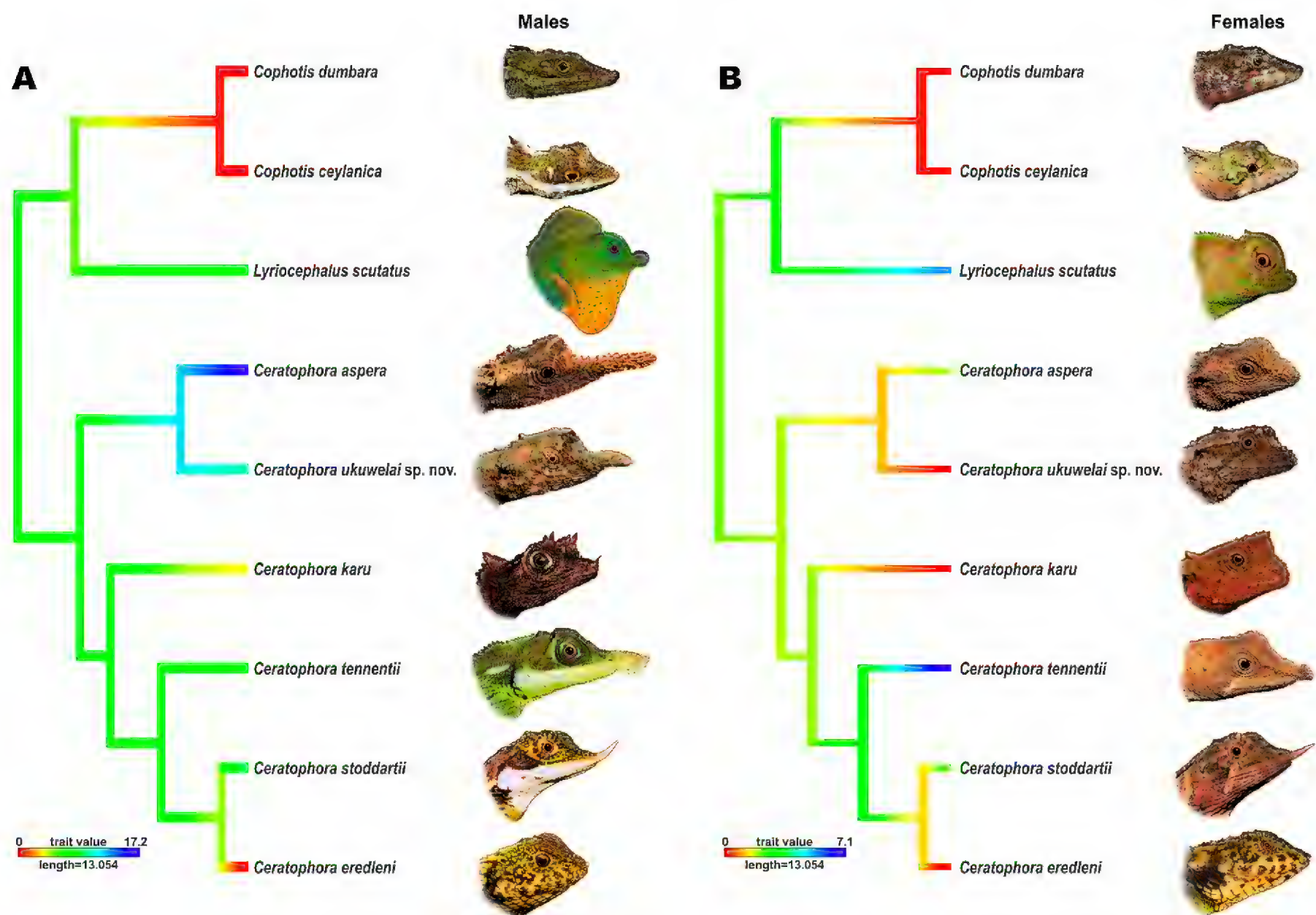


Fig. 4. Relative rostral appendage (RAL/SVL) evolution among members of the Sri Lankan agamids (genera *Ceratophora*, *Lyriocephalus*, and *Cophotis*). See Table 2 for RAL/SVL data. Colors of branches correspond to average RAL/SVL values in males (A) and females (B); thumbnails show profiles of the respective lizard species (not to scale). *Photos by Sanoj Wijayasekara, Sanjaya Kanishka, and Suranjan Karunarathna.*

Salgala Forest represents a divergent mtDNA lineage sister to *C. aspera*, with a species-level divergence of this population in the *ND2* gene ($p = 9.6\%$). The early (late Miocene) split between these two lineages, along with a number of diagnostic morphological characters which distinguish *Ceratophora* sp. from Salgala Forest from *C. aspera* and from other congeners (see **Comparisons**), suggest that *Ceratophora* sp. from Salgala Forest represents a currently undescribed species new to science which is described below.

Ceratophora ukuwelai sp. nov.

Figs. 5–6; Tables 5–6.

urn:lsid:zoobank.org:act:3F34CFA5-59BA-4B28-B9D4-7A16B69CB95E

Holotype. NMSL 2020.05.01, adult female, 37.9 mm SVL, collected from rainforest flow neighboring a stream, Salgala Forest, Kegalle District, Sri Lanka (7.120219°N, 80.251892°E, WGS1984; elevation 242 m; around 1100 h) on 22 August 2019 by Suranjan Karunarathna and Anslem de Silva.

Paratype. NMSL 2020.05.02, adult female, 36.4 mm SVL, collected from rainforest flow neighboring a stream,

Salgala forest, Kegalle District, Sri Lanka (7.074361°N, 80.249797°E, WGS1984; elevation 269 m; around 1000 h) on 22 August 2019 by Suranjan Karunarathna and Anslem de Silva.

Diagnosis. The new species is assigned to the genus *Ceratophora* on the basis of phylogenetic data and by having a rostral appendage developed in males, absent in females; tympanum covered with skin; nuchal crest indistinct; dorsal crest absent; tail not prehensile; gular fold comparatively reduced; and scales on flanks heterogeneous, some scales greatly enlarged. *Ceratophora ukuwelai* sp. nov. can be readily distinguished from its congeners by a combination of the following morphological and meristic characteristics: rostral appendage complex, comprising several scales; maximum SVL 37.9 mm; trunk relatively long (TRL/SVL ratio 51.4–52.6%) with relatively short fore-body (SA/TRL ratio 90.2–90.9%); nuchal crest feebly defined; squamosal process present; dorsum with heterogeneous, keeled scales, intermixed with smooth flat scales; almost all scales on head, body, limbs, and tail bearing 1–18 mechanoreceptive pores (in a single scale), each pore with a sensory seta; 5–7 enlarged, keeled scales present on body flanks; nine supraciliary scales; 40–44 paravertebral scales; 72–77 midbody scales; 72–75

Table 3. Uncorrected *p*-distances (percentage) between the *ND2* mtDNA gene sequences (below the diagonal), estimate errors (above the diagonal), and intraspecific genetic *p*-distance (on the diagonal) of Sri Lankan agamid species of the genera *Ceratophora*, *Cophotis*, and *Lyriocephalus*.

Species	1	2	3	4	5	6	7	8	9
1 <i>Ceratophora stoddartii</i>	0.8	0.5	1.0	1.1	1.0	1.1	1.0	1.1	1.1
2 <i>Ceratophora erdeleni</i>	3.8	—	1.0	1.1	1.0	1.1	1.1	1.2	1.2
3 <i>Ceratophora tennentii</i>	10.7	12.5	—	1.2	1.2	1.1	1.2	1.1	1.2
4 <i>Ceratophora karu</i>	18.1	18.7	16.5	—	1.2	1.5	1.2	1.1	1.2
5 <i>Ceratophora aspera</i>	18.8	20.4	18.7	20.6	—	0.9	1.0	1.1	1.0
6 <i>Ceratophora ukuwelai</i> sp. nov.	20.6	21.5	19.2	23.0	9.6	—	1.1	1.1	1.1
7 <i>Cophotis ceylanica</i>	19.8	20.8	20.5	21.2	22.9	24.4	—	0.7	1.0
8 <i>Cophotis dumbara</i>	20.1	21.2	20.6	20.0	22.5	24.3	4.7	—	1.1
9 <i>Lyriocephalus scutatus</i>	22.4	22.8	22.1	23.1	22.7	24.6	17.9	18.7	—

midventral scales. The new species is also clearly distinct from all other congeners in *ND2* gene sequences (divergence over 9.6%).

Description of holotype. An adult female, 37.9 mm SVL and 42.6 mm original TAL (Fig. 5), in a good state of preservation (however, 15 mm of the tail was used for the molecular work). For counts and measurements of the holotype see Tables 5–6. Body slender, relatively long (TRL/SVL ratio 51.4%). Head relatively large (HL/SVL ratio 30.0% and HL/TRL ratio 58.5%), broad (HW/SVL ratio 17.8% and HW/HL ratio 59.3%), partly depressed (HD/SVL ratio 14.4% and HD/HL ratio 47.8%), and distinct from neck. Snout relatively long (ES/HW ratio 56.8% and ES/HL ratio 33.7%), less than twice orbit diameter (OD/ES ratio 84.4%), more than half length of jaw (ES/JL ratio 51.5%), snout slightly concave in lateral view; orbit relatively large (OD/HL ratio 28.4%), pupil rounded; orbit length slightly greater than IV digit of manus (OD/DLM IV ratio 100.6%); supraocular rim moderately developed; supraciliaries uplifted; two rows of scales separate orbit from supralabials; interorbital distance is shorter than snout length (IO/ES ratio 49.2%), shorter than head length (IO/HL ratio 16.6%), eye to nostril distance greater than the interorbital distance (EN/IO ratio 102.6%).

Dorsal, lateral, and ventral surfaces of the head, trunk, and tail with keeled scales intermixed with smooth heterogeneous, small and large scales, each scale with at least one or more pores (up to 18) bearing a sensory seta; rostral horn absent, rostral scales very small; snout convex, scales on snout keeled and raised, smaller than those on interorbital and occipital regions; canthus scales present, 11/10 keeled conical scales from eye to nostril; nasal scale large, nostril rounded and located in the middle of an undivided nasal scale, not in contact with supralabials; scales of the interorbital region heterogeneous, intermixed with smooth scales; palpable squamosal process present. Nuchal crest not prominent, 1–3 pointed and ridged scales on the neck; supralabials 12/12 keeled, infralabials 13/12 keeled, becoming

smaller towards the gape. Two scale rows separate orbit from supralabials. Sharp and conical tubercles present both on the sides of the neck and around the gape; tympanum hidden under skin; enlarged, keeled, and flat scales present on tympanum area; 44 paravertebral scales, four diamond shaped markings with three black

Table 4. The calculated Pagel's λ for SVL, head, and rostral appendage measurements for males and females of Sri Lankan agamids of the genera *Ceratophora*, *Cophotis* and *Lyriocephalus*. Asterisks denote *p*-values indicating significant differences of Pagel's λ from zero (**p* < 0.05; ***p* < 0.01). Residual RAL and Residual Log RAL represent the residuals after regression of RAL and Log RAL on SVL and log SVL, respectively. PGLS means that phylogenetic signal in the residual error was simultaneously estimated with the phylogenetic regression parameters (the regression formula is in parentheses).

Character	Pagel's λ	
	Males	Females
SVL	1.10**	1.05*
Log SVL	1.10**	1.04*
JL	1.10**	1.02
Log JL	1.10**	1.04
HD	1.11**	1.09**
Log HD	1.09*	1.09*
RAL	0.00	1.04
Log RAL	0.11	0.00
Max RAD	1.06	1.09*
Log Max RAD	0.00	0.00
Relative RAL (to SVL)	0.46	0.00
Relative RAD (to SVL)	0.93	1.07
Residual RAL	0.00	1.09
Residual Log RAL	0.07	0.00
PGLS(RAL~SVL)	0.00	0.00
PGLS(RAD~SVL)	0.00	0.00
PGLS(LogRAL~LogSVL)	0.00	0.00
PGLS(LogRAD~LogSVL)	0.00	0.00

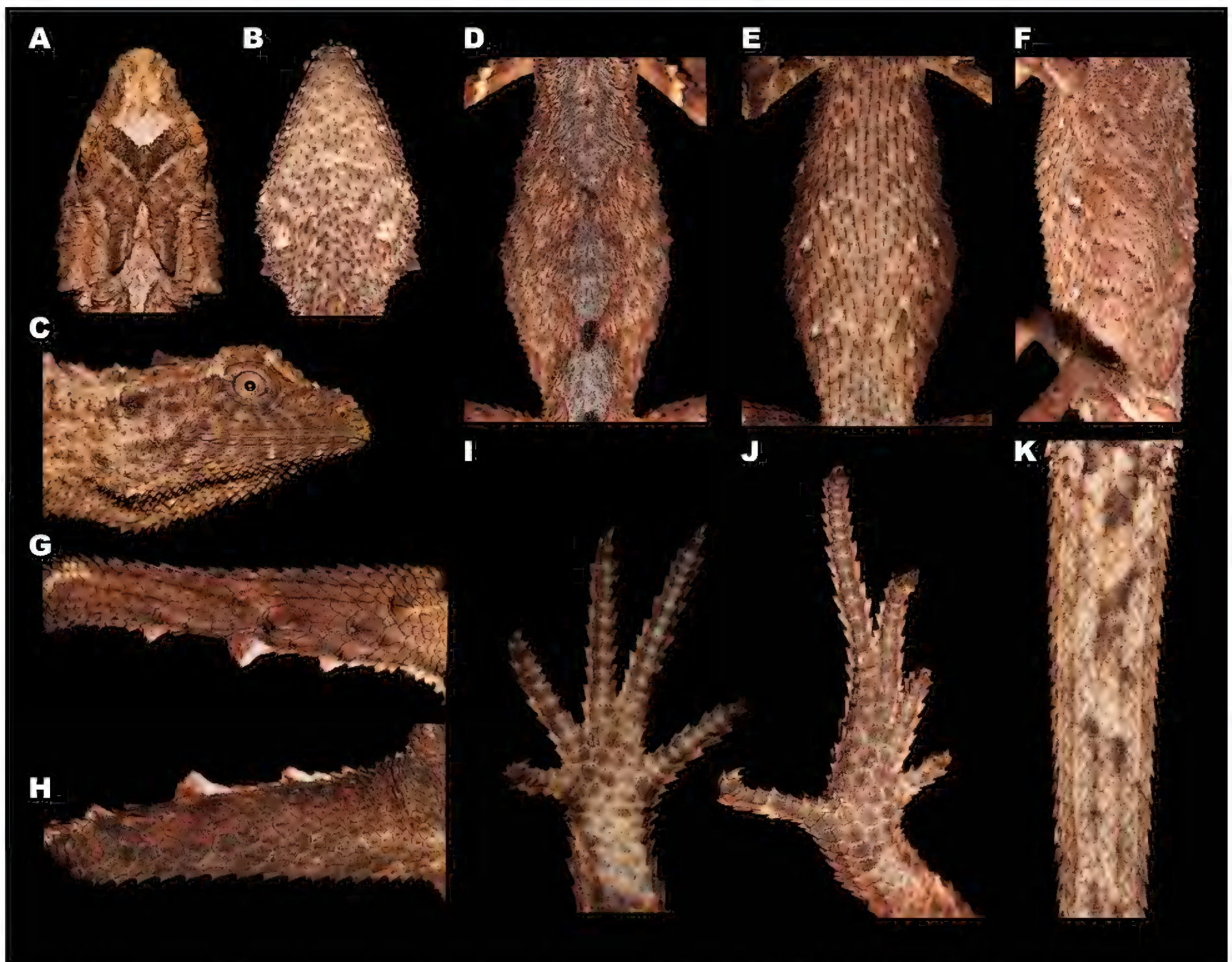


Fig. 5. Morphology of *Ceratophora ukuwelai* sp. nov. holotype, adult female (NMSL 2020.05.01). (A) Head in dorsal view; (B) head in ventral view; (C) head in lateral view; (D) heterogeneous scales on dorsal surface of trunk; (E) keeled ventral scales; (F) lateral surfaces of trunk showing heterogeneous scales; (G–H) dorsal and ventral surfaces of femur showing sharp spines; (I) subdigital lamellae on manus; (J) subdigital lamellae on pes; (K) hexagonal-shaped subcaudals. Photos by Suranjan Karunaratna.

dots present on vertebral line; 77 midbody scales; lateral scales irregular and keeled, intermixed with 5/6 enlarged scales on the flanks.

Ventral surfaces covered with keeled scales, each scale with one or more mechanoreceptive pores; mental semicircular in shape, small, posteriorly in contact with three small postmentals (smaller than naris, chin scales, and rostral scales), in contact with the 1st infralabial. Gular fold short and its length is approximately 22.6% of SVL, but dispersed and its depth is approximately 60.3% of HD. Ventral scales pentagonal, subimbricate, ventral scales larger than chin scales, dorsal scales, and lateral scales, 75 ventral scales; keeled scales around vent and base of tail; no precloacal or femoral pores; original tail of holotype longer than the snout-vent length (TAL/SVL ratio 112.3%), heterogeneous scales on the dorsal aspect of the tail directed backwards, spine-like scales present on tail; subcaudals keeled and small, subrhomboidal, arranged in a single median series. Forelimbs long, slender, upper arm longer than lower arm (LAL/SVL ratio 15.9% and UAL/SVL ratio 17.8%); hindlimbs long, tibia slightly shorter than the femur (TBL/SVL ratio 22.8% and

FEL/SVL ratio 23.7%). Anterior, dorsal, posterior, and ventral surfaces of forelimbs and hindlimbs with strongly keeled and less imbricate scales; anterior surfaces twice as large as those of the other surfaces of both limbs; posterior edges of femur and tibia with six large, conical scales.

Dorsal and ventral surfaces of manus and pes with keeled granules; dorsal surfaces of digits with granular scales. Digits elongate and slender with inflected distal phalanges, all bearing slightly recurved claws. Subdigital lamellae on digits entire, notched; lamellae on manus (left/right): digit I (7/6), digit II (9/9), digit III (13/12), digit IV (13/13), digit V (9/8); total lamellae on pes (left/right): digit I (6/6), digit II (8/7), digit III (8/8), digit IV (15/14), digit V (7/7); interdigital webbing absent; relative length of left manual digits: I (1.8 mm), V (2.1 mm), II (2.5 mm), III (2.9 mm), IV (3.2 mm); relative length of left pedal digits: I (1.6 mm), II (2.2 mm), III (2.8 mm), V (3.3 mm), IV (5.7 mm).

Variation. Measurements and morphological characters of the type series are given in Tables 5–6. The female paratype is generally similar to the holotype in body

Table 5. Morphometric data for two syntypes of *Ceratophora aspera* and two types of *C. ukuwelai* **sp. nov.** from Sri Lanka (all in mm).

Measurement	<i>C. aspera</i>		<i>C. ukuwelai</i> sp. nov.	
	BMNH. 1946.8.30.52	BMNH. 1946.8.30.51	NMSL. 2020.05.01	NMSL. 2020.05.02
	Male	Female	Female	Female
SVL	28.1	36.5	37.9	36.4
TRL	11.6	16.2	19.5	19.1
HL	8.5	9.7	11.4	11.1
HW	5.5	6.1	6.8	6.6
HD	4.6	5.4	5.5	5.4
RAL	2.1	-	-	-
SA	13.2	17.5	17.7	17.2
JL	5.7	5.8	7.5	7.4
TAL (original)	40.7	42.3	42.6	41.2
OD	3.1	2.9	3.2	3.2
EN	2.1	1.9	1.9	1.9
ES	3.2	2.1	3.8	3.6
SN	1.2	0.9	1.3	1.3
IO	2.2	2.4	1.9	1.9
UAL	5.2	6.3	6.8	6.7
LAL	4.4	5.3	6.0	5.9
PAL	3.5	4.8	6.5	6.5
DLM (i)	1.5	1.9	1.8	1.8
DLM (ii)	2.1	2.5	2.5	2.4
DLM (iii)	2.6	2.9	2.9	2.8
DLM (iv)	2.9	3.1	3.2	3.1
DLM (v)	1.8	2.2	2.1	2.2
FEL	7.3	7.5	9.0	8.9
TBL	6.6	7.4	8.7	8.5
HEL	7.9	9.2	9.5	9.3
DLP (i)	1.1	1.5	1.6	1.5
DLP (ii)	1.6	2.3	2.2	2.1
DLP (iii)	2.6	3.8	2.8	2.7
DLP (iv)	4.3	6.5	5.7	5.5
DLP (v)	2.4	2.9	3.3	3.2

proportions and coloration; the SVL of adult female specimens in the type series of *Ceratophora ukuwelai* **sp. nov.** ($n = 2$) ranges from 36.4 to 37.9 mm; enlarged flank scales 5–7; supralabials 12–13; infralabials 11–12; postmentals 3–4; interorbital 9–10; canthal scales 10–11; total lamellae on digit of the manus: digit I (6–7), digit II (8–9), digit III (12–13), digit IV (12–13), digit V (8–9); total lamellae on digit of the pes: digit I (6–7), digit II (7–8), digit III (7–8), digit IV (14–16); paravertebral granules 40–44; midbody scales 72–77; ventral scales 72–75 (see Tables 5–6). Because the holotype and paratype of the new species are females, sexual dimorphism could

not be determined. However, a single male specimen of *Ceratophora ukuwelai* **sp. nov.** was recorded at the type locality and photographed in life (Fig. 6B). Male specimen possessed long (RAL/SVL ratio 11.26%) complex rostral appendage, comprised of numerous keeled acuminate scales, including posterostral scales and a pointed enlarged scale on the top.

Color of living specimens. In life, dorsum of head, body, and limbs generally grey-brown (Fig. 6); forehead with white blotch, interorbital area with a ‘Y’ shaped brown marking, occiput area with a ‘W’ shaped dark marking;

Table 6. Meristic data of two syntypes of *Ceratophora aspera* and two types of *C. ukuwelai* **sp. nov.** from Sri Lanka.

Measurement	<i>C. aspera</i>		<i>C. ukuwelai</i> sp. nov.	
	BMNH. 1946.8.30.52	BMNH. 1946.8.30.51	NMSL. 2020.05.01	NMSL. 2020.05.02
	Male	Female	Female	Female
FLSP (L/R)	10/9	11/10	5/6	7/6
SUP (L/R)	10/9	10/11	12/12	13/12
INF (L/R)	10/11	10/9	12/12	11/12
PM	4	4	3	4
SUS (L/R)	12	14	9	9
INOS	13	15	10	9
CAS (L/R)	14/13	12/13	11/10	11/10
TLM (i) (L/R)	7/8	6/5	7/6	6/6
TLM (ii) (L/R)	9/10	6/8	9/9	8/9
TLM (iii) (L/R)	12/13	10/12	13/12	12/12
TLM (iv) (L/R)	14/12	12/11	13/13	13/12
TLM (v) (L/R)	9/9	8/7	9/8	8/8
PS	58	52	44	40
MBS	61	57	77	72
MVS	92	95	75	72
TLP (i) (L/R)	6/5	7/6	6/6	7/6
TLP (ii) (L/R)	7/8	6/8	8/7	7/7
TLP (iii) (L/R)	13/12	10/9	8/8	8/7
TLP (iv) (L/R)	16/17	14/14	15/14	16/15
TLP (v) (L/R)	9/8	7/8	7/7	7/7

four grey diamond-shaped vertebral markings with black dots. Tail generally brown with faded zigzag markings. Two brown postorbital stripes on each side with striped labials (Fig. 5). Chin, gular, and ventral scales dirty white mixed with red-brown. Dorsal surface of upper and lower arm with white ring around. Posterior side of femur with white longitudinal spine line, tibia with white ring around. Iris copper-orange; pupil black. Inner surfaces of mouth cavity bluish-grey. A male specimen (not collected) showed generally similar but slightly darker coloration than the female type (Fig. 6B).

Color of preserved specimens. After preservation in ethanol for one year, coloration pattern of type specimens resembles that observed in life. Dorsally specimens turned dark brown with four distinct diamond-shaped markings on vertebrae; interorbital area with a Y-shaped dark marking; both limbs with dirty white rings. Ventral surfaces turned grey-brown.

Etymology. The specific epithet is a Latinized eponym in the masculine genitive singular, honoring evolutionary biologist and herpetologist Dr. Kanishka Ukuwela (Rajarata University) for his invaluable contribution to biodiversity studies and conservation in Sri Lanka.

Suggested common names. Ukuwelas' Rough-horn Lizard (English), Ukuwelage ralu-ang katussa (Sinhala).

Comparisons with other Sri Lankan species. The new species, *Ceratophora ukuwelai* **sp. nov.**, readily differs from *Ceratophora aspera* by the presence of fewer supraciliary scales (9 versus 12–14), fewer paravertebral scales (40–44 versus 52–58), greater midbody scales (72–77 versus 57–61), fewer ventral scales (72–75 versus 92–95), trunk relatively long (TRL/SVL ratio 51.4–52.6% versus 41.3–44.5%), and fore-body relatively short (SA/TRL ratio 90.2–90.9% versus 107.6–113.9%). Differs from *Ceratophora erdeleni* by the presence of a long, complex, and rough rostral appendage in males (versus short, simple, and smooth rostral appendage), lateral scales keeled (versus lateral scales smooth), relatively small bodied, average SVL of adults (37 mm versus 80 mm), found in lowland wet zone (below 300 m versus above 900 m). Differs from *Ceratophora karu* by the presence of long and rough rostral appendage in males (versus short, pointed, and relatively smooth rostral appendage), no prominent and conical shaped superciliary (versus very prominent and conical shaped superciliary presents), squamosal process present (versus squamosal process absent), found in

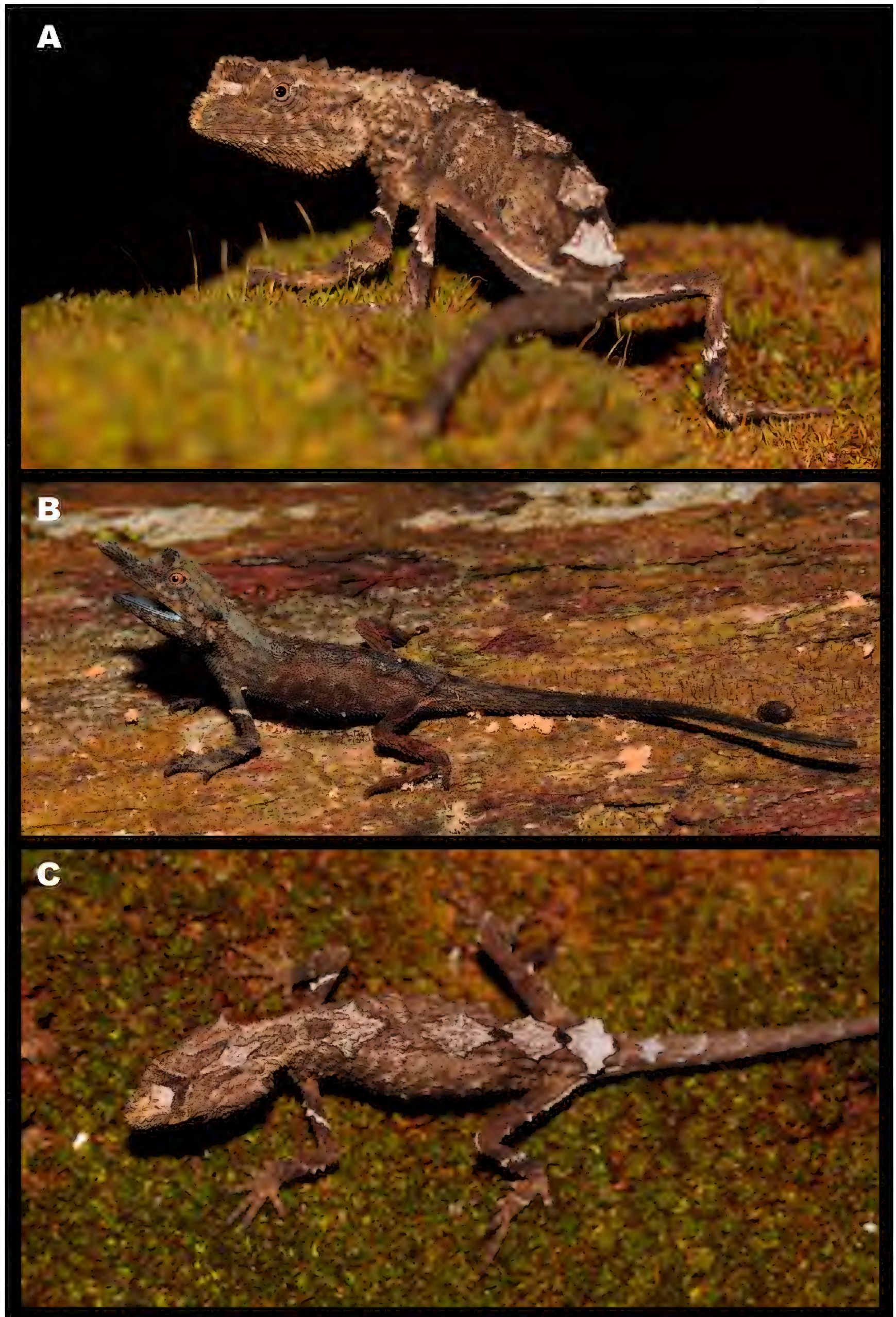


Fig. 6. *Ceratophora ukuwelai* sp. nov. in life *in-situ*. (A) Female holotype (NMSL 2020.05.01) in dorsolateral view; (B) male specimen (not collected) in dorsolateral aspect showing rostral appendage; (C) female paratype (NMSL 2020.05.02) in dorsal view. Photos by Suranjan Karunaratna and Sanjaya Kanishka.



Fig. 7. Habitat of *Ceratophora ukuwelai* **sp. nov.** at type locality in Salgala Forest, Kegalle District, Sri Lanka. **(A)** General view of Salgala Forest; **(B)** microhabitat of the new species inside the dense forest with good canopy cover and thick leaf litter. Photos by Suranjan Karunarathna.

lowland wet zone (below 300 m versus above 900 m). Differs from *Ceratophora stoddarti* by the presence of long, complex, and rough rostral appendage in males (versus long, simple, and smooth rostral appendage), lateral scales keeled (versus lateral scales smooth), relatively small bodied, average SVL of adults (37 mm versus 80 mm), found in lowland wet zone (below 300 m versus above 800 m). Differs from *Ceratophora tennentii* by the presence of rough and relatively round shaped rostral appendage in males (versus smooth and laterally flattened rostral appendage), lateral scales keeled (versus lateral scales smooth), relatively small bodied, average SVL of adults (37 mm versus 80 mm), found in lowland wet zone (below 300 m versus above 800 m).

Distribution and natural history. The type locality, Salgala Forest (7.109631–7.129028°N, 80.243444–80.263494°E; Kegalle District, Sabaragamuwa Province), is located in the lowland at elevations of 120–325 m asl. The area falls within the northern border of the wet bioclimatic zone, where tropical evergreen rainforests comprise the dominant vegetation type (Gunatilleke and Gunatilleke 1990). The forest acreage is approximately 150 ha and Salgala forest is isolated from other forest massifs by the Kelani River and Maha River valleys, numerous perennial middle-order streams, and human modified cultural landscapes such as tea plantations. The mean annual rainfall in the area varies between 2,500 and 3,500 mm, most of it is received during the southwest monsoon (May–September), while the mean

annual temperature is around 29.2 °C. Salgala is rich in tall rainforest trees and the forest floor contains thick leaf litter. Numerous smaller streams are present within the type locality. *Ceratophora ukuwelai* **sp. nov.** appears to be an elusive and rare species in Salgala as only five individuals were recorded during 10 field excursions (nearly 500 person-hours). Specimens of the new species were recorded on the forest floor in dense forest patches with thick and wet leaf litter under dense canopy cover (Fig. 7). The microhabitat of *Ceratophora ukuwelai* **sp. nov.** was a poorly illuminated (light intensity: 455–687 Lux), relatively moist, canopy-shaded (relative humidity: 72–84% and canopy cover: 70–85%), and relatively warm environment (substrate temperature: 27.7–28.2 °C) by the time of our survey. The new species was recorded in sympatry with several other agamid lizard species, including *Calotes calotes* (Linnaeus, 1758), *Calotes liolepis* Boulenger, 1885, *Calotes versicolor* (Daudin, 1802), and *Otocryptis wiegmanni* Wagler, 1830.

Conservation status. Application of the IUCN Red List criteria indicates that *C. ukuwelai* **sp. nov.** has to be considered a Critically Endangered (CR) species due to having an area of occupancy (AOO) < 10 km² (four locations, 0.12 km² in total assuming a 100 m radius around the georeferenced locations) and an extent of occurrence (EOO) < 100 km² (0.26 km²) in Kegalle District, Sabaragamuwa Province of southwestern Sri Lanka [Applicable criteria B2-b (iii)] (IUCN Standards and Petitions Subcommittee 2016).

Discussion

In 1864, Albert Günther described a new horned lizard, which he named *Ceratophora aspera*, from Ceylon (historical name of Sri Lanka) based on the collections sent to London by Hugh Cuming (Günther 1864; Amarasinghe et al. 2009). However, Günther mentioned that *C. aspera* probably came from the montane part of Sri Lanka, likely from the same source as the specimens of *C. stoddartii* and *C. tennentii* (Günther 1861, 1864). Most of the species described by Günther from Cuming's collection are now known to be restricted to the south-west wet zone of Sri Lanka (Pethiyagoda 2007; Meegaskumbura et al. 2008; Amarasinghe et al. 2009; Sudasinghe and Pethiyagoda 2019), suggesting that this area likely corresponds to the type locality of *C. aspera*. Additionally, we examined the two syntypes of *C. aspera* housed in British Museum of Natural History (female BMNH.1946.8.30.51, and male BMNH.1946.8.30.52) [Fig. 8] and obtained the morphometric and meristic characters from these specimens for comparison. The Salgala population described herein as *Ceratophora ukuwelai* **sp. nov.** originates from the northern border of the wet bioclimatic zone (Kegalle District), and so *C. aspera* populations are restricted to the southern part of the wet bioclimatic zone (Galle District). However, this



Fig. 8. Syntypes of *Ceratophora aspera* Günther, 1864 in lateral view: BMNH.1946.30.51 (female, above) and BMNH.1946.30.52 (male, below). Photo by Colin McCarthy (BMNH).

study revealed a number of taxonomically important morphological differences between these populations, and demonstrated that they are also genetically distinct (p -distance 9.6%). The southern and northern populations of this complex are separated by 115 km direct distance (Fig. 1) and by the valleys of the Attanagalu, Kelani, Kalu, and Gin rivers and a number of mountain ridges. These geographical barriers have likely impeded gene flow, resulting in reproductive isolation. The molecular dating analysis suggested that *Ceratophora ukuwelai* **sp. nov.** and *C. aspera* have been separated presumably since the late Miocene. These results continue to underscore the high degree of site-specific endemism in isolated forest patches within the lowland areas of wet bioclimatic zone in Sri Lanka (e.g., Manamendra-Arachchi and Pethiyagoda 2005; Meegaskumbura and Manamendra-Arachchi 2005; Agarwal et al. 2017; Karunaratna et al. 2019; Danushka et al. 2020) and the need for additional field research throughout these insular habitats.

The most recent discovery of new species in the genus *Ceratophora* was the description of *C. erdeleni* and *C. karu* from Rakwana Hills over two decades ago (Pethiyagoda and Manamendra-Arachchi 1998). Most *Ceratophora* species are rare, range-restricted endemics. At present, *C. tennentii* is restricted to the Knuckles Hills, and *C. stoddartii* occupies the Central Highlands; while *C. erdeleni* and *C. karu* are restricted to the Rakwana Hills (Fig. 1). *Ceratophora aspera* was

once thought to be a more widely ranging species with a patchy distribution across the lowland tropical rainforests within the wet bioclimatic zone of Sri Lanka. This study demonstrates that the northernmost portion of its range actually harbors a new species, *Ceratophora ukuwelai* **sp. nov.**, while *C. aspera sensu stricto* appears to be restricted to the southern part of the wet bioclimatic zone (Fig. 1). Further studies of morphological and genetic variation across the isolated populations of *C. aspera* are needed to assess the true taxonomic diversity and extend of distribution of *Ceratophora* species in Sri Lanka.

In the present paper we recommend that *Ceratophora ukuwelai* **sp. nov.** be listed as a Critically Endangered (CR) species. The infrequent encounter rates of this species in its habitat and continuing habitat loss are the primary reasons for our conservation status assessment. In addition, Sri Lanka's southwestern lowland rainforests are severely fragmented; as such, edge effects and concomitant micro-environmental changes and subsidized predation risk could further endanger this species. The threats to agamid lizards would appear to stem largely from habitat loss and fragmentation. The impacts of fragmentations could also be exacerbated by the fact that many important montane forest fragments are surrounded by vegetable and tea plantations. Worse yet, vegetable cultivation in Sri Lanka involves the intensive and indiscriminate application of pesticides (Karunaratna et al. 2017), which can reduce the

agamids' prey base. In addition, the bioconcentration of pesticides in lizards has been well documented in other tropical realms (Campbell and Campbell 2000, 2002; Khan and Law 2005). Highways also pose a threat to animals, not only by means of habitat fragmentation, but also by resulting in direct mortality in terms of incidental roadkills. The asphalt surfaces of these highways reach thermally intolerable levels, which could induce physiological stress. The exotic pet trade and alien invasive species are growing threats for Sri Lankan lizards (Karunaratna and Amarasinghe 2013; Janssen and de Silva 2019). In addition, predation by feral or domestic cats can also result in considerable mortality among agamids (Arnaud et al. 1993; Tyler et al. 2016). Further studies on the natural history and behavior of endemic lizards of Sri Lanka are essential for better planning and implementation of scientific conservation and management programs (Karunaratna et al. 2011). The promotion of ecological and behavioral studies in schools and universities is required for assessing habitat fragmentation and human impacts on Sri Lankan endemic agamid lizards (Manamendra-Arachchi and Liyanage 1994; Karunaratna and Amarasinghe 2013). Further development of public awareness workshops and conservation action plans are necessary for the conservation of agamid species. Reducing road kills at road crossings and migration routes and further development of public awareness through the organization of workshops are important steps for the implementation of a conservation action plan for Sri Lankan agamid conservation (Karunaratna et al. 2013). We are also unaware of any substantial *ex-situ* efforts in the captive breeding of agamids. Sri Lanka's zoological and botanical gardens should explore the feasibility of such efforts.

Our updated phylogeny for Sri Lankan agamids allowed us to re-analyze patterns of possible evolution of the rostral appendage—a bizarre morphological structure characteristic to the genus *Ceratophora* (Fig. 4). A high phylogenetic signal in body size traits in Sri Lankan agamids was found, which is not surprising for such morphological traits and was demonstrated earlier for a number of lizard groups (Freckleton et al. 2002; Ashton 2004; Brandt and Navas 2011; Oufiero et al. 2011; Grizante et al. 2012; Hertz et al. 2013; Openshaw and Keogh 2014; Wegener et al. 2014; Mesquita et al. 2016). Pagel's $\lambda = 1$ implied that the evolution of these traits followed Brownian motion (Freckleton et al. 2002). Surprisingly, however, we have not detected phylogenetic signal in rostral appendage measurements. This could be related to the insufficient number of species in our analysis, but the high values of Pagel's λ in body size indicate that our sample size is sufficient to detect at least a high phylogenetic signal in traits. In the case of *Ceratophora*, phylogenetically closely related species may have opposing states for rostral appendage characters. Our analysis thus suggests that rostral appendage length and

depth have evolved largely independently of phylogeny. For example, the two sister species with minimal genetic divergence between them may show the presence (*C. stoddartii*) or absence (*C. erdeleni*) of RA in both sexes (Fig. 4; Table 2). Our reconstruction of ancestral states in RA evolution suggested that rostral ornamentation was likely present both in males and females of the common ancestor of the *Ceratophora* – *Lyriocephalus* – *Cophotis* clade (Fig. 4). The RA was subsequently lost in both sexes of *Cophotis* and *C. erdeleni*, reduced in both sexes of *C. karu* and females of *C. aspera* and *C. ukuwelai* **sp. nov.**, and enlarged in males of *C. aspera* and *C. ukuwelai* **sp. nov.**, and in females of *Lyriocephalus* and *C. tennentii*. However, the absence of phylogenetic signal in the evolution of RA structures in Sri Lankan agamids reported here makes the goal of robustly reconstructing the evolutionary history of this feature even more challenging.

Rostral appendages exhibit great variability in morphology, dimorphism, and ontogeny among the members of the *Ceratophora* – *Lyriocephalus* – *Cophotis* clade (Fig. 4). Several studies have addressed the problem of rostral appendage origin and evolution in *Ceratophora* species using parsimony (Schulte et al. 2002) and Bayesian (Johnston et al. 2012) approaches. Schulte et al. (2002) noted that the profound morphological differences observed among rostral structures of *C. aspera*, *C. stoddartii*, and *C. tennentii*, and the fact that these species do not form a clade, suggest three independent origins of these unusual ornaments in *Ceratophora*. Johnston et al. (2012) provided further morphological, allometric, and phylogenetic evidence suggesting that rostral appendages evolved three times within three separate lineages of *Ceratophora*. Johnston et al. (2012) further argued that in the case of *C. tennentii* it was likely driven by the natural selection for crypsis, while in *C. aspera* and *C. stoddartii* the independent origin of RA might be a result of sexual selection. Whiting et al. (2015) analyzed sexual dimorphism in RA parameters and coloration in *C. tennentii*, and did not find a correlation between these characters with bite force or body condition in this species. However, Whiting et al. (2015) assumed that RAL still might be a target of sexual selection and may serve as a cue used by females to assess some aspect of male quality. Our results generally agree with the hypothesis of Johnston et al. (2012) and provide further evidence that rostral appendages in Sri Lankan agamids likely evolved by several mechanisms, and more readily than in any other group of lizards. Further detailed studies of phylogeny and diversity within the *Ceratophora* – *Lyriocephalus* – *Cophotis* clade, along with research on the natural history of the comprising species, and a more thorough anatomical comparison of rostral structures, are needed to generate a more detailed and robust scenario of rostral appendage evolution in this group.

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Key to Sri Lankan species of genus *Ceratophora*

1a. Rostral appendage simple, restricted to rostral scale alone.....	2
1b. Rostral appendage complex, comprising more scales than rostral alone.....	3
2a. Rostral appendage rudimentary in both sexes (appendage is shorter than eye-nostril distance).....	<i>C. erdeleni</i>
2b. Rostral appendage prominent in males (appendage is longer than eye-nostril distance).....	<i>C. stoddartii</i>
3a. Rostral appendage laterally compressed.....	<i>C. tennentii</i>
3b. Rostral appendage not laterally compressed.....	4
4a. Squamosal process absent, represented by an enlarged scale.....	<i>C. karu</i>
4b. A prominent squamosal process present.....	5
5a. Trunk length is less than half of SVL and snout to axilla length is longer than trunk length (52–58 paravertebrals and 92–95 ventrals).....	<i>C. aspera</i>
5b. Trunk length is more than half of SVL and snout to axilla length is shorter than trunk length (40–44 paravertebrals and 72–75 ventrals).....	<i>Ceratophora ukuwelai</i> sp. nov.

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Literature Cited

Agarwal I, Biswas S, Bauer AM, Greenbaum E, Jackman TR, de Silva A, Batuwita S. 2017. Cryptic species, taxonomic inflation, or a bit of both? New species phenomenon in Sri Lanka as suggested by a phylogeny of dwarf geckos (Reptilia, Squamata, Gekkonidae, *Cnemaspis*). *Systematics and Biodiversity* 15: 1–13.

Amarasinghe AAT, Manthey U, Stöckli E, Ineich I, Kullander SO, Tiedemann F, McCarthy C, Gabadage DE. 2009. The original descriptions and figures of Sri

Lankan agamid lizards (Squamata: Agamidae) of the 18th and 19th centuries. *Taprobanica* 1: 2–15, 4 pls.

Arnaud G, Rodríguez A, Ortega-Rubio A, Álvarez-Cárdenas S. 1993. Predation by cats on the unique endemic lizard of Socorro Island (*Urosaurus auriculatus*), Revillagigedo, Mexico. *Ohio Journal of Science* 93: 101–104.

Ashton KG. 2004. Comparing phylogenetic signal in intraspecific and interspecific body size datasets. *Journal of Evolutionary Biology* 17: 1,157–1,161.

Bahir MM, Surasinghe TD. 2005. A conservation assessment of the agamid lizards of Sri Lanka. *Raffles Bulletin of Zoology* 12: 407–412.

Blomberg SP, Garland T. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation, and comparative methods. *Journal of Evolutionary Biology* 15: 899–910.

Bossuyt F, Meegaskumbura M, Beenaerts N, Gower DJ, Pethiyagoda R, Roelants K, Mannaert A, Wilkinson M, Bahir MM, Manamendra-Arachchi K, et al. 2004. Local endemism within the Western Ghats-Sri Lanka Biodiversity Hotspot. *Science* 306: 479–481.

Bossuyt F, Meegaskumbura M, Beenaerts N, Gower DJ, Pethiyagoda R, Roelants K, Mannaert A, Wilkinson M, Schneider CJ, Bahir MM, et al. 2005. Biodiversity in Sri Lanka and the Western Ghats - response. *Science* 308: 199.

Brandt R, Navas CA. 2011. Life-history evolution on Tropicurinae lizards: influence of lineage, body size, and climate. *PLoS ONE* 6: e20040.

Campbell KR, Campbell TS. 2000. Lizard contaminant data for ecological risk assessment. *Reviews of Environmental Contamination and Toxicology* 15: 39–116.

Campbell KR, Campbell TS. 2002. A logical starting point for developing priorities for lizard and snake ecotoxicology: a review of available data. *Environmental Toxicology and Chemistry* 21: 894–

- 898.
- Cincotta RP, Wisnewski J, Engelman R. 2000. Human populations in the biodiversity hotspots. *Nature* 404: 990–992.
- Cooray PG. 1967. An introduction to the geology of Ceylon. *Spolia Zeylanica* 31: 1–324.
- Danushka AD, Kanishka AS, Amarasinghe AAT, Vogel G, Seneviratne SS. 2020. A new species of *Dendrelaphis* Boulenger, 1890 (Reptilia: Colubridae) from the wet zone of Sri Lanka with a redescription of *Dendrelaphis bifrenalis* (Boulenger, 1890). *Taprobanica* 9: 30–36.
- de Silva A, Ukuwela K. 2020. *A Naturalist's Guide to the Reptiles of Sri Lanka*. John Beaufoy Publishing Ltd., Oxford, United Kingdom. 176 p.
- de Silva A. 2006. Current status of the reptiles of Sri Lanka. Pp. 134–163 In: *Fauna of Sri Lanka: Status of Taxonomy, Research, and Conservation*. Editor, Bambaradeniya CMB. IUCN Sri Lanka, Colombo, Sri Lanka. 308 p.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29(8): 1,969–1,973.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Freckleton RP, Harvey PH, Pagel M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist* 160: 712–726.
- Gibson C, de Silva A, Tognelli MF, Karunarathna S. (Editors). 2020. *Assess to Plan: Conservation Action Planning for the Snakes and Lizards of Sri Lanka*. IUCN Conservation Planning Specialist Group, Apple Valley, Minnesota, USA. 74 p.
- Gray JE. 1833–1835. *Illustrations of Indian Zoology; Chiefly Selected from the Collection of Major-General Hardwicke, F.R.S., Volume II*. Adolphus Richter, London, United Kingdom.
- Greenbaum E, Bauer AM, Jackman TR, Vences M, Glaw F. 2007. A phylogeny of the enigmatic Madagascan geckos of the genus *Uroplatus* (Squamata: Gekkonidae). *Zootaxa* 1493: 41–51.
- Greller AM, Balasubramaniam S. 1980. A preliminary floristic-climatic classification of the forests of Sri Lanka. *Sri Lanka Forester* 14: 163–170.
- Grismer JL, Schulte JA, Alexander A, Wagner P, Travers SL, Buehler MD, Welton LJ, Brown RM. 2016. The Eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian dragon lizards. *BMC Evolutionary Biology* 16: 1–12.
- Grizante MB, Brandt R, Kohlsdorf T. 2012. Evolution of body elongation in gymnophthalmid lizards: relationships with climate. *PLoS ONE* 7: e49772.
- Gunatileke IAUN, Gunatileke CVS. 1990. Distribution of floristic richness and its conservation in Sri Lanka. *Conservation Biology* 4: 21–31.
- Gunawardene NR, Daniels AED, Gunatilleke IAUN, Gunatilleke CVS, Karunakaran PV, Nayak KG, Prasad S, Puyravaud P, Ramesh BR, Subramanian KA, et al. 2007. A brief overview of the Western Ghats-Sri Lanka Biodiversity Hotspot. *Current Science* 93: 1,567–1,572.
- Günther A. 1861. In Tennent JE, *Sketches of the Natural History of Ceylon with Narratives and Anecdotes Illustrative of the Habits and Instincts of the Mammalia, Birds, Reptiles, Fishes, Insects, & including a Monograph of the Elephant and a Description of the Modes of Capturing and Training It*. Longman, Green, Longman, and Roberts, London, United Kingdom. 580 p.
- Günther A. 1864. *The Reptiles of British India*. The Ray Society, London, United Kingdom. 452 p.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Helgen KM, Groves CP. 2005. Biodiversity in Sri Lanka and Western Ghats. *Science* 308: 199.
- Hertz PE, Arima Y, Harrison A, Huey RB, Losos JB, Glor RE. 2013. Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution* 67: 2,101–2,113.
- Hillis DM, Moritz C, Mable BK. 1996. *Molecular Systematics*. 2nd edition. Sinauer Associates, Sunderland, Massachusetts, USA. 655 p.
- Huelsenbeck JP, Hillis DM. 1993. Success of phylogenetic methods in the four-taxon case. *Systematic Biology* 42: 247–264.
- IUCN Standards and Petitions Subcommittee. 2016. Guidelines for using the IUCN Red List Categories and Criteria. Version 12. Prepared by the Standards and Petitions Subcommittee. Available: <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [Accessed: 15 August 2020].
- Janssen J, de Silva A. 2019. The presence of protected reptiles from Sri Lanka in international commercial trade. *Traffic Bulletin* 31: 9–15.
- Johnston GR, Lee M, Surasinghe TD. 2012. Morphology and allometry suggest multiple origins of rostral appendages in Sri Lankan agamid lizards. *Journal of Zoology* 289: 1–9.
- Karunarathna DMSS, Henkanaththegedara SM, Amarasinghe AAT, de Silva A. 2013. Impact of vehicular traffic on herpetofaunal mortality in a savannah forest, eastern Sri Lanka. *Taprobanica* 5: 111–119.
- Karunarathna S, Amarasinghe T. 2013. Behavioral ecology and microhabitat use by *Lyriocephalus scutatus* (Linnaeus, 1758): a monotypic genus in Sri Lanka (Reptilia: Agamidae: Draconinae), with notes on the taxonomy. *Russian Journal of Herpetology* 20: 1–15.
- Karunarathna S, Henkanaththegedara S, Gabadage D, Botejue M, Madawala M, Surasinghe T. 2017. Ecology and demography of the Critically Endangered

- Kandian Torrent Toad *Adenomus kandianus*: a long-lost endemic species of Sri Lanka. *Oryx* 51: 619–626.
- Karunaratna S, Poyarkov NA, de Silva A, Madawala M, Botejue M, Gorin VA, Surasinghe T, Gabadage D, Ukuwela KDB, Bauer AM. 2019. Integrative taxonomy reveals six new species of day geckos of the genus *Cnemaspis* Strauch, 1887 (Reptilia: Squamata: Gekkonidae) from geographically isolated hill forests in Sri Lanka. *Vertebrate Zoology* 64: 247–298.
- Karunaratna S, Pradeep G, Peabotuwage I, de Silva M. 2011. First report on the ovipositional behavior of *Calotes nigrilabris* Peters, 1860 (Reptilia: Sauria: Agamidae) from the Central massif of Sri Lanka. *Russian Journal of Herpetology* 18: 111–118.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3,059–3,066.
- Khan MZ, Law FC. 2005. Adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish, amphibians, and reptiles: a review. *Proceedings of the Pakistan Academy of Sciences* 42: 315–323.
- Kozlov A, Darriba D, Flouri T, Morel B, Stamatakis A. 2018. RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *BioRxiv* 447110.
- Kurita T, Kojima Y, Hossman MY, Nishikawa K. 2020. Phylogenetic position of a bizarre lizard, *Harpesaurus*, implies the co-evolution between arboreality, locomotion, and reproductive mode in Draconinae (Squamata: Agamidae). *Systematics and Biodiversity* 18: 675–687.
- Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ. 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* 14: 91–104.
- Macey JR, Schulte JA, Larson A. 2000a. Evolution and phylogenetic information content of mitochondrial genomic structural features illustrated with acrodont lizards. *Systematic Biology* 49: 257–277.
- Macey JR, Schulte JA, Larson A, Ananjeva NB, Wang Y, Pethiyagoda R, Rastegar-Pouyani N, Papenfuss TJ. 2000b. Evaluating trans-Tethys migration: An example using acrodont lizard phylogenetics. *Systematic Biology* 49: 233–256.
- Manamendra-Arachchi K, Pethiyagoda R. 2005. The Sri Lankan shrub-frogs of the genus *Philautus* Gistel, 1848 (Ranidae: Rhacophorinae), with description of 27 new species. *Raffles Bulletin of Zoology* 12: 163–303.
- Manamendra-Arachchi K, Liyanage S. 1994. Conservation of the agamid lizards of Sri Lanka with illustration of the extant species. *Journal of South Asian Natural History* 1: 77–96.
- Meegaskumbura M, Bossuyt F, Pethiyagoda R, Manamendra-Arachchi K, Bahir M, Milinkovitch MC, Schneider CJ. 2002. Sri Lanka: an amphibian hotspot. *Science* 298: 379.
- Meegaskumbura M, Manamendra-Arachchi K. 2005. Description of eight new species of shrubfrogs (Ranidae: Rhacophorinae: *Philautus*) from Sri Lanka. *Raffles Bulletin of Zoology* 12: 305–338.
- Meegaskumbura M, Senevirathne G, Manamendra-Arachchi K, Pethiyagoda R, Hanken J, Schneider CJ. 2019. Diversification of shrub frogs (Rhacophoridae, *Pseudophilautus*) in Sri Lanka: timing and geographic context. *Molecular Phylogenetics and Evolution* 132: 14–24.
- Meegaskumbura M, Silva A, Maduwage K, Pethiyagoda R. 2008. *Puntius reval*, a new barb from Sri Lanka (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters* 19: 141–152.
- Mesquita DO, Costa GC, Colli GR, Costa TB, Shepard DB, Vitt LJ, Pianka ER. 2016. Life-history patterns of lizards of the world. *The American Naturalist* 187: 689–705.
- MOE-SL. 2012. *The National Red List of Sri Lanka: Conservation Status of the Fauna and Flora*. Biodiversity Secretariat, Ministry of Environment, Colombo, Sri Lanka. 451 p.
- Molina-Venegas R, Rodríguez MÁ. 2017. Revisiting phylogenetic signal: strong or negligible impacts of polytomies and branch length information? *BMC Evolutionary Biology* 17: 53.
- Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffrers K, Thuiller W. 2012. How to measure and test phylogenetic signal. *Methods in Ecology and Evolution* 3: 743–756.
- Openshaw GH, Keogh JS. 2014. Head shape evolution in monitor lizards (*Varanus*): interactions between extreme size disparity, phylogeny, and ecology. *Journal of Evolutionary Biology* 27: 363–373.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2018. R Core Team caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. Available: <https://cran.r-project.org/web/packages/caper/index.html> [Accessed: 1 June 2020].
- Oufiero CE, Gartner GEA, Adolph SC, Garland T. 2011. Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* 65: 3,590–3,607.
- Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.
- Pethiyagoda R, Manamendra-Arachchi K. 1998. A revision of the endemic Sri Lankan agamid lizard genus *Ceratophora* Gray, 1835, with description of two new species. *Journal of South Asian Natural History* 3: 1–50.
- Pethiyagoda R. 2007. *Pearls, Spices, and Green Gold: an Illustrated History of Biodiversity Exploration in Sri Lanka*. WHT Publications, Colombo, Sri Lanka.

241 p.

- Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Pyron RA, Kandambi HKD, Hendry CR, Pushpamal V, Burbrink FT, Somaweera R. 2013. Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. *Molecular Phylogenetics and Evolution* 66: 969–978.
- R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org/> [Accessed: 1 June 2020].
- Rambaut A, Suchard M, Xie W, Drummond A. 2014. Tracer v. 1.6. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom. Available: <http://tree.bio.ed.ac.uk/software/tracer/> [Accessed: 1 June 2020].
- Revell LJ. 2010. Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution* 1: 319–329.
- Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.
- Revell LJ. 2013. Two new graphical methods for mapping trait evolution on phylogenies. *Methods in Ecology and Evolution* 4: 754–759.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1,572–1,574.
- RStudio Team. 2018. RStudio: integrated development for R. RStudio, Inc., Boston, Massachusetts, USA.
- Schulte II JA, Macey JR, Pethiyagoda R, Larson A. 2002. Rostral horn evolution among agamid lizards of the genus *Ceratophora* endemic to Sri Lanka. *Molecular Phylogenetics and Evolution* 22: 111–117.
- Somaweera R, Somaweera N. 2009. *Lizards of Sri Lanka: a Color Guide with Field Keys*. Edition Chimaira, Frankfurt am Main, Germany. 303 p.
- Stacklies W, Redestig H, Scholz M, Walther D, Selbig J. 2007. pcaMethods—a bioconductor package providing PCA methods for incomplete data. *Bioinformatics* 23: 1,164–1,167.
- Sudasinghe H, Pethiyagoda R. 2019. A commentary on the taxonomic review of Sri Lankan *Devario* by Batuwita et al. 2017 (Teleostei: Danionidae). *Zootaxa* 4543: 421–430.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2,725–2,729.
- Tyler RK, Winchell KM, Revell LJ. 2016. Tails of the city: caudal autotomy in the tropical lizard, *Anolis cristatellus*, in urban and natural areas of Puerto Rico. *Journal of Herpetology* 50: 435–441.
- Uetz P, Cherkikh S, Shea G, Ineich I, Campbell PD, Doronin IV, Rosado J, Wynn A, Tighe KA, McDiarmid R, et al. 2019b. A global catalog of primary reptile type specimens. *Zootaxa* 4695: 438–450.
- Wang K, Che J, Lin S, Deepak V, Aniruddha DR, Jiang K, Jin J, Chen H, Siler CD. 2019. Multilocus phylogeny and revised classification for mountain dragons of the genus *Japahura* sl. (Reptilia: Agamidae: Draconinae) from Asia. *Zoological Journal of the Linnean Society* 185: 246–267.
- Wegener JE, Gartner GEA, Losos JB. 2014. Lizard scales in an adaptive radiation: variation in scale number follows climatic and structural habitat diversity in *Anolis* lizards. *Biological Journal of the Linnean Society* 113: 570–579.
- Whiting MJ, Noble DW, Somaweera R. 2015. Sexual dimorphism in conspicuousness and ornamentation in the enigmatic leaf-nosed lizard *Ceratophora tennentii* from Sri Lanka. *Biological Journal of the Linnean Society* 116: 614–625.
- Williams EE. 1979. South American anoles: the species groups. 2. The proboscis anoles (*Anolis leavis* group). *Breviora* 449: 1–18.



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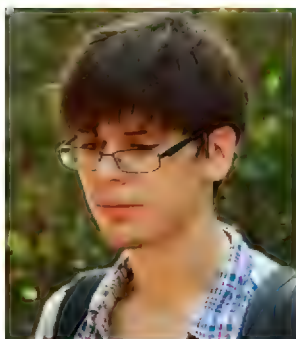
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Appendix 1. Comparative materials examined.

Ceratophora aspera: BMNH.1946.30.51 (female), BMNH.1946.30.52 (male), WHT.0178 (male), WHT.1366 (female), WHT.1369 (male), WHT.1370 (female), WHT.1371 (male), WHT.2170 (male), WHT.1396 (female), WHT.1400 (male).

Ceratophora erdeleni: BMNH.1996.448 (holotype male), BMNH.1996.450 (paratype male), BMNH.1996.449 (paratype female), WHT.1328 (male), WHT2070 (male), WHT.2172 (female), WHT2175 (male).

Ceratophora karu: BMNH.1996.445 (holotype male), BMNH.1996.446 (female), BMNH.1996.447 (male), WHT.2065 (male), WHT.2067 (male), WHT.2068 (female).

Ceratophora stoddartii: BMNH.1946.8.27.37 (male), WHT.0209 (female), WHT.1170 (male), WHT.1327 (male), WHT.1700 (male), WHT.1702 (female).

Ceratophora tennentii: BMNH.1946.8.27.33 (syntype male), WHT.0103 (male), WHT.0114 (female), WHT.1350 (male).

Cophotis ceylanica: ZMB.4240 (lectotype), WHT.0177 (female), WHT.0516 (male), WHT.0645 (male), WHT.5817 (female), WHT.5818 (female), WHT.5819 (male).

Cophotis dumbara: CMS.2006.85.01 (male holotype), CMS.2006.85.02 (female paratype), WHT.6788 (male), WHT.6948 (male), WHT.6789 (female).

Lyriocephalus scutatus: WHT.0175 (female), NMSL.0462 (male), NMSL.0471 (male), NMSL.0485 (female).



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A new species of terrestrial-breeding frog of the genus *Psychrophrynella* (Anura: Strabomantidae) from the Cordillera de Vilcabamba, southeastern Peru

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Abstract.—A new frog of the genus *Psychrophrynella* is described based on specimens from the Cordillera de Vilcabamba, in the department of Cusco in southeastern Peru. The new species inhabits the humid puna and is only known from its type locality in Challcha, near the road between Vilcabamba and Pampaconas, at 3,707 m asl. This new taxon is assigned to the genus *Psychrophrynella* based on a narrowest genetic distance of 16S rRNA with *P. glauca* (8.3%) and the presence of a fold-like tubercle on the inner edges of the tarsus. The description of *Psychrophrynella vilcabambensis* sp. nov. is based on three individuals. This new species can be differentiated from other members of the genera *Psychrophrynella* and *Noblella* by the combination of the following characters: light reddish-brown to tan coloration on the dorsum with dark brown markings, the presence of a thoracic fold, ulnar tubercles, a tubercle on the heel, three tubercles on outer edge of tarsus, and toes with lateral fringes. The SVL of male and female specimens are 16.5 and 16.6 mm, respectively.

Keywords. Amphibia, Andes, Cusco, Holoadeninae, *Noblella*, *Psychrophrynella vilcabambensis* sp. nov.

Resumen.—Describimos una nueva rana del género *Psychrophrynella* de la Cordillera de Vilcabamba, en el departamento de Cusco al sudeste del Perú. La nueva especie habita la puna húmeda y sólo se conoce de su localidad tipo en Challcha, cerca de la carretera entre Vilcabamba y Pampaconas, a 3,707 m snm. El nuevo taxón se asigna al género *Psychrophrynella*, basándose en la distancia genética de 16S ARNr más estrecha con *P. glauca* (8.3%), y la presencia de un tubérculo alargado similar a un pliegue en el borde interior del tarso. *Psychrophrynella vilcabambensis* sp. nov. fue descrita en base a tres individuos. Esta nueva especie se puede diferenciar de otros miembros de los géneros *Psychrophrynella* y *Noblella* por la siguiente combinación de caracteres: coloración marrón rojiza clara a marrón bronceada en el dorso con manchas marrón oscuras, presencia de pliegue torácico, tubérculos cubitales, un tubérculo en el talón, tres tubérculos en el borde exterior del tarso y dedos con rebordes laterales. El SVL del macho y de la hembra es 16.5 y 16.6 mm, respectivamente.

Palabras clave. Andes, Anfibia, Cusco, Holoadeninae, *Noblella*, *Psychrophrynella vilcabambensis* sp. nov.

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Introduction

The subfamily Holoadeninae Hedges, Duellman, and Heinicke, 2008 was constructed based on molecular data, and includes the genera *Barycholos*, *Bryophryne*,

Euparkerella, *Holoaden*, *Microkayla*, *Noblella*, *Psychrophrynella*, and *Qosqophryne* (Catenazzi et al. 2020; Heinicke et al. 2018). *Psychrophrynella bagrecito* Hedges, Duellman, and Heinicke, 2008 is the type species of the genus *Psychrophrynella*, proposed by Hedges et al.

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(2008). In the same study, Hedges et al. (2008) include one Peruvian and 18 Bolivian species in the genus *Psychrophrynella*. Subsequent studies increased the diversity of the genus to 22 species distributed in humid puna of the Andes of southeastern Peru and northwestern Bolivia (De la Riva and Burrowes 2014; De la Riva and Aparicio 2016). However, in 2017, additional molecular studies by De la Riva et al. (2017) divided the genus *Psychrophrynella* into two genera, *Psychrophrynella* and the new genus *Microkayla*. In this new genus, these authors included 24 species from Bolivia and three from Peru. Moreover, De la Riva et al. (2017) supported the monophyly and validity of the genus *Psychrophrynella*, reducing the number of species in this genus to three (*P. bagrecito*, *P. chirihampatu*, and *P. usurpator*), while the other species were moved to the genus *Microkayla*. Later, Catenazzi and Ttito (2018) described *P. glauca* from Peru.

Thus, the Peruvian endemic genus *Psychrophrynella* currently contains four formally described species (*P. bagrecito*, *P. usurpator*, *P. chirihampatu*, and *P. glauca*). This genus is distributed along the eastern slopes of southeastern Peruvian Andes, between the departments of Cusco and Puno at 2,225–3,400 m asl, where these frogs are typically found in the leaf litter and under stones and terrestrial mosses (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Lynch 1986).

Despite the advancements in the systematics and taxonomy of the subfamily Holoadeninae (De la Riva 2020; De la Riva et al. 2017; Heinicke et al. 2018), our understanding of some lineages is still precarious. One example is the uncertainty regarding the phylogenetic relationship between *Psychrophrynella* and *Noblella* (Catenazzi and Ttito 2016, 2018; Catenazzi et al. 2020). The genus *Noblella* is polyphyletic and includes two divergent lineages: one containing five species with distribution ranges from central Peru to Ecuador, and the other with four species from southeastern Peru (Catenazzi and Ttito 2019; Reyes-Puig et al. 2019; Santa Cruz et al. 2019). The type species of *Noblella* (*N. peruviana*) and *Psychrophrynella* (*P. bagrecito*) are both distributed in southeastern Peru, but genetic sequences are not available for these two species and the synapomorphies that support *Noblella* and *Psychrophrynella* are inconclusive (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Santa Cruz et al. 2019). Herein, a morphological description is provided for a new species of terrestrial-breeding frog from the Cordillera de Vilcabamba, department of Cusco in southeastern Peru. This species was recognized as a new species in some earlier studies, being listed as *Psychrophrynella* sp. by Lehr and Catenazzi (2010) and Catenazzi et al. (2020).

Materials and Methods

Data collection. Specimens were collected by hand and euthanized by application of 8% benzocaine paste on the

dorsal and ventral regions. Tissue samples (muscle) were stored in 2 mL cryogenic tubes filled with 96% ethanol, and specimens were fixed in 10% formalin and preserved in 70% ethanol. Specimens were deposited in the herpetological collection of the Museo de Biodiversidad del Peru (MUBI).

Morphology. The description of morphological characters of *Psychrophrynella* follows Duellman and Lehr (2009), and Catenazzi and Ttito (2016, 2018). The taxonomic classification follows Heinicke et al. (2018). Morphometric measurements were taken using a digital caliper and a stereoscope. Abbreviations of measurements are as follows: snout-vent length (SVL), tibia length (TL), foot length (FL, distance from the proximal margin of inner metatarsal tubercle to tip of Toe IV), head length (HL, from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), eye diameter (ED), tympanum diameter (TY), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), and eye-nostril distance (E-N, straight line distance between the anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to post axially from I–IV and I–V, respectively. To determine the lengths of Toes III and V, both toes were pressed against Toe IV, and the lengths of fingers I and II were determined by pressing these fingers against each other. The variation in coloration in life is based on the field notes and photographs by the third author (LM). This new species was compared with the descriptions as published in the literature for the four valid and formally described species of the genus *Psychrophrynella* (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Lynch 1986), and five species of the genus *Noblella* from southern Peru (Catenazzi and Ttito 2019; Catenazzi et al. 2015; Lehr and Catenazzi 2009; Noble 1921; Santa Cruz et al. 2019). Examined material is listed in **Appendix 1**.

DNA extraction, amplification, and sequencing.

Genomic DNA was extracted from the voucher specimen MUBI 13485 using the QIAGEN DNeasy Blood and Tissue extraction kit following the manufacturer's protocol. Fragments of the mitochondrial long subunit rRNA gene (16S) were amplified by Polymerase Chain Reaction (PCR) with the following conditions: an initial 2 min at 93 °C, followed by 35 cycles of 30 sec at 95 °C, 1 min at 42 °C, 1.5 min at 72 °C, and a final extension step of 6 min at 72 °C. The primers used were 16Sar (CGC CTG TTT ATC AAA AAC AT) and 16Sbr (CCG GTC TGA ACT CAG ATC ACG T) [Palumbi et al. 1991]. Purified PCR products were sent to Macrogen Inc. (Seoul, Republic of Korea) for sequencing in both directions with the amplification primers. Raw sequence chromatographs for sequences generated in this study were edited using AliView 1.14 (Larsson 2014). One new gene sequence of this locus was produced with GenBank accession number MT818174.

Table 1. GenBank codes for sequences of the species of the subfamily Holoadeninae used in this study.

Species and voucher specimens	GenBank accession (16S)	Source
<i>Barycholos ternetzi</i> CFBHT 04408	KU495152	Lyra et al. 2016
<i>Bryophryne bakersfield</i> MUBI 6022	MF186341	De la Riva et al. 2017
<i>Bryophryne cophites</i> AC 270.07	KY652641	von May et al. 2017
<i>Bryophryne hanssaueri</i> MUSM 27567	KY652642	von May et al. 2017
<i>Bryophryne nubilosus</i> MUSM 27882	KY652643	von May et al. 2017
<i>Bryophryne phuyuhampatu</i> CORBIDI 18226	MF419256	Catenazzi et al. 2017
<i>Holoaden luederwaldti</i> CFBHT 07810	KU495249	Lyra et al. 2016
<i>Microkayla chilina</i> MNCN 43774	MF186416	De la Riva et al. 2017
<i>Microkayla iatamasi</i> MNCN 42054	MF186368	De la Riva et al. 2017
<i>Microkayla katantika</i> CBF 6012	MF186380	De la Riva et al. 2017
<i>Noblella lochites</i> KU 177356	EU186699	Hedges et al. 2008
<i>Noblella losamigos</i> MUSA 6973	KY652644	von May et al 2017
<i>Noblella madreseiva</i> CORBIDI 15770	MN056356	Catenazzi and Ttito 2019
<i>Noblella myrmecoides</i> QCAZ 40180	JX267542	Canedo and Haddad 2012
<i>Noblella pygmaea</i> MUSM 24536	KY652645	von May et al. 2017
<i>Noblella thiuni</i> CORBIDI 18723	MK072732	Catenazzi and Ttito 2019
<i>Phrynopus peruanus</i> MUSM 38316	MG896582	von May et al. 2018
<i>Psychrophrynella chirihampatu</i> MUBI 14664	KU884560	Catenazzi and Ttito 2016
<i>Psychrophrynella glauca</i> CORBIDI 18729	MG837565	Catenazzi and Ttito 2018
<i>Psychrophrynella usurpator</i> AC186.09	KY652662	von May et al. 2017
<i>Psychrophrynella vilcabambensis</i> sp. nov. MUBI 13485	MT818174	This study
<i>Psychrophrynella</i> sp. MUSM 27619	MT437065	Catenazzi et al. 2020

Genetic distances. Uncorrected *p*-distances were estimated using the 16S rRNA mitochondrial gene, comparing the new species to some representative species of the genera *Barycholos*, *Bryophryne*, *Holoaden*, *Microkayla*, *Noblella*, *Phrynopus*, and *Psychrophrynella*, which were available in GenBank (Table 1). The DNA sequences were aligned in MUSCLE (Edgar 2004) and uncorrected *p*-distances were estimated in MEGAX (Kumar et al. 2018). Following Catenazzi and Ttito (2016, 2018), phylogenetic analyses were not performed because taxonomic uncertainty exists in the genera *Noblella* and *Psychrophrynella*; and molecular information about the type species, *Noblella peruviana* and *Psychrophrynella bagrecito*, are needed for taxonomic resolution.

Results

Generic placement. The new species is placed in the genus *Psychrophrynella* (Hedges et al. 2008) on the basis of morphological and molecular data. The main diagnostic phenotypic traits of *Psychrophrynella* are: (1) tympanic membrane and annulus differentiated (annulus and membrane visible beneath skin); (2) tips of digits narrow and rounded, not expanded, lacking circumferential grooves and pads; and (3) inner edge of tarsus bearing a prominent, elongate, sigmoid-shaped or fold-like tubercle (De la Riva et al. 2017). These aforementioned characteristics are shared by the new species. Additionally, analyses of the

uncorrected *p*-distances for 16S rRNA showed that the new species has wide genetic distances from all species that were compared (Table 2), the narrowest being with *P. glauca* (8.3%) and *N. thiuni* (9.4%).

Taxonomy

Psychrophrynella vilcabambensis **sp. nov.**
Psychrophrynella sp. Lehr and Catenazzi 2010: 317
Psychrophrynella sp. MUSM 27619 Catenazzi et al. 2020: 10.

urn:lsid:zoobank.org:act:C56F4DBA-5594-4069-BE91-0376E705542C

Holotype. MUBI 13485, an adult male (Fig. 1) from Challcha (13°05'44"S, 73°01'37.7"W) [WGS84], 3,707 m asl, district of Vilcabamba, province of La Convención, department of Cusco, Peru; collected on 8 August 2016, by F.P. Condori, L. Mamani, and J.A. Delgado.

Paratypes. Two specimens: one adult female, MUBI 13486 (Fig. 2A–B), and one juvenile, MUBI 13484 (Fig. 2C–D), same data as holotype.

Diagnosis. *Psychrophrynella vilcabambensis* **sp. nov.** is characterized by having: (1) skin on dorsum shagreen with small warts, coalescing into linear ridges at midbody; dorsolateral fold visible on half of the body



Fig. 1. Photographs in life of the holotype of *Psychrophrynella vilcabambensis* **sp. nov.**, adult male MUBI 13485 (SVL = 16.5 mm.). (A–B) Dorsolateral views; (C) ventral view.

and ending posteriorly in a sacral tubercle; skin on venter smooth, discoidal, and thoracic fold present; (2) tympanic membrane not differentiated, anteroventral part of tympanic annulus barely visible below skin; (3) snout short, rounded in dorsal view and in profile; (4) upper eyelid narrower than IOD, bearing small tubercles; cranial crests absent; (5) dentigerous processes of vomers absent; (6) vocal slits present; nuptial pads absent; (7) fingers lacking lateral fringes; Finger I shorter than Finger II; tips of digits rounded, not expanded laterally; (8) ulnar tubercles present; (9) heel with one tubercle; inner edge of tarsus bearing an elongate, oblique fold-like tubercle; outer edge of tarsus with some tubercles; (10) inner metatarsal tubercle prominent elliptical, 1.25 times larger than ovoid outer metatarsal tubercle; supernumerary plantar tubercles small, poorly defined; (11) toes with lateral fringes; webbing absent; Toe V slightly

shorter than or equal to Toe III; tips of digits rounded, not expanded; (12) dorsum light reddish brown to tan, with or without a pale middorsal line extending from tip of snout to the cloaca, and with dark brown markings, inside of which there are dermal protuberances; interorbital blotch present; flanks dark reddish brown; chest and throat dark brown with moderate or abundant pale gray flecks; palmar and plantar surfaces dark brown with tiny pale gray flecks; belly and legs grayish brown with pale gray flecks; (13) SVL of males 16.5 mm (based on a single adult specimen), SVL of females 16.63 mm (based on a single adult specimen) [Table 3].

Comparative diagnosis. The new species differs morphologically from species of *Noblella* in southern Peru (*N. losamigos*, *N. madrevelva*, *N. peruviana*, *N. pygmaea*, and *N. thiuni*) based on discoidal fold. It is also different from *N. losamigos*, *N. madrevelva*, *N. peruviana*, and *N. pygmaea* due to the absence of elongate acuminate toe tips. Relative to all species of *Psychrophrynella*, it differs in having unique characters such as light reddish-brown to tan coloration on dorsal surfaces with dark brown marks and in presenting a thoracic fold, toes with lateral fringes (Fig. 3A), one tubercle on the heel and some tubercles on the outer edge of the tarsus, and ulnar tubercles (Fig. 3B). Morphologically, *P. vilcabambensis* **sp. nov.** is similar to *P. chirihampatu* in having a large fold-like tubercle on the inner edge of the tarsus, a prominent elliptical inner metatarsal tubercle larger than the ovoid outer metatarsal tubercle, and shagreen dorsum skin with small warts forming linear ridges at the middorsum. However, *P. vilcabambensis* **sp. nov.** can be distinguished from *P. chirihampatu* by having a visible discoidal fold (not visible in *P. chirihampatu*), small upper eyelid tubercles (lacking in *P. chirihampatu*), and an inner metatarsal tubercle 1.25 times larger than the outer metatarsal tubercle (versus 1.5 times). From *P. bagrecito*, the new species differs by having smooth (areolate) skin on venter, short snout (moderately long), tarsus with an elongate fold-like tubercle (short), inner metatarsal tubercle larger than outer metatarsal tubercle (equal), poorly defined small supernumerary plantar tubercles (lacking), and Toe V slightly shorter or equal to Toe III (Toe V shorter than Toe III). From *P. glauca*, it differs by having a dorsolateral fold (absent), short snout (snout very short), small upper eyelid tubercles (lacking), tarsus elongate with fold-like tubercle (short), and inner metatarsal tubercle larger than outer metatarsal tubercle (equal). From *P. usurpator*, the new species differs by having shagreen dorsum skin (smooth), small upper eyelid tubercles (lacking), inner metatarsal tubercle larger than outer metatarsal tubercle (inner metatarsal tubercle same size as outer metatarsal tubercle), and Toe V slightly shorter than or equal to Toe III (Toe V shorter than Toe III).

Description of holotype. Adult male (SVL 16.5 mm); head narrower than the body, its length 28.30% of SVL;



Fig. 2. Photographs in life of paratypes of *Psychrophrynella vilcabambensis* sp. nov. (A–B) Dorsolateral and ventral views of adult female, MUBI 13486 (SVL = 16.63 mm); (C–D) dorsolateral and ventral views of juvenile MUBI 13484 (SVL = 12.99 mm).

head slightly wider than long, HW 117.8% of HL; HW 33.3% of SVL; snout short, rounded in dorsal and lateral views, ED 38.97% of HL, its diameter 1.6 times as large as its distance from the nostril; nostrils not protuberant, close to snout, directed laterally; canthus rostralis concave in dorsal view, slightly convex in profile; loreal region flat; lips rounded; upper eyelids with small tubercles; EW 62.6% of IOD; interorbital region flat, lacking cranial crests; E–N distance 62.1% of ED; supratympanic fold absent; tympanic membrane not differentiated, anteroventral part of tympanic annulus visible below skin; postrictal tubercles present. Choanae round, small; dentigerous processes of vomers and vomerine teeth absent; the tongue covers almost the entire floor of the mouth, and it is large and ovoid.

Skin on dorsum shagreen with small warts, which are equally distributed on the dorsum, at middorsum these warts conform linear ridges; dorsolateral folds present only anteriorly and terminate posteriorly in a sacral tubercle; skin on flanks shagreen; venter smooth; pectoral and discoidal fold present; cloaca not protuberant, cloacal region with small tubercles. Ulnar tubercles present; circular outer palmar tubercle approximately the same length but twice the width of oval thenar tubercle; supernumerary palmar tubercles present; subarticular tubercles prominent, rounded in ventral and lateral view; fingers lacking lateral fringes, not webbed; relative lengths of fingers $3 > 4 > 2 > 1$; tips of digits bulbous, not expanded laterally.

Hindlimbs moderately long, TL 44.4% of SVL; FL 51.5% of SVL, upper surface shagreen with moderately small tubercles; posterior surfaces smooth; heel with one tubercle; inner edge of tarsus bearing a large, oblique fold-like tubercle, outer edge of tarsus with tubercles; elliptical inner metatarsal tubercle larger than ovoid outer metatarsal tubercle; plantar supernumerary tubercles weakly defined; subarticular tubercles rounded in ventral view and ovoid in profile view; toes with lateral fringes, not webbed; toe tips weakly pointed, not expanded laterally; relative lengths of toes $4 > 3 > 5 > 2 > 1$ (Fig. 2A).

Measurements of holotype (in mm). SVL 16.5, TL 7.33, FL 8.49, HL 4.67, HW 5.5, ED 1.82, TY 0.89, IOD 2.19, EW 1.37, IND 1.8, E–N 1.13.

Coloration of holotype in life. Dorsal surfaces of head, body, and extremities reddish brown, with dark brown markings bordered by a poorly defined cream stripe. Lateral surface of the head with three dark brown labial bars, the middle one in contact with the eye; and a dark reddish-brown stripe, extending from the tip of the snout to the border of the eye, crossing above the tympanum and extending to the insertion of the forelimb. This stripe becomes redder and lighter as it approaches the insertion of the forelimb. Iris dark reddish brown with abundant black reticulations and gold pallid stripe on

Table 2. Uncorrected *p*-distances for 16S rRNA sequences between *Psychrophrynella vilcabambensis* **sp. nov.** and related taxa in the subfamily Holadeninae. The genetically most similar species are highlighted in blue.

	<i>Barycholos ternetzi</i>	<i>Bryophryne bakersfield</i>	<i>Bryophryne cophites</i>	<i>Bryophryne hanssaueri</i>	<i>Bryophryne nubilosus</i>	<i>Bryophryne phuyuhampatu</i>	<i>Holoaden luederwaldti</i>	<i>Microkayla chilina</i>	<i>Microkayla iatamasi</i>	<i>Microkayla katanitika</i>	<i>Noblella lochites</i>	<i>Psychrophrynella vilcabambensis</i> sp. nov.	<i>Noblella losamigos</i>	<i>Noblella myrmecoides</i>	<i>Noblella pygmaea</i>	<i>Noblella madreselva</i>	<i>Noblella thiuni</i>	<i>Psychrophrynella chirihampatu</i>	<i>Psychrophrynella glauca</i>	<i>Psychrophrynella usurpator</i>	<i>Phrynopus peruanus</i>
<i>Bryophryne bakersfield</i>	19.8																				
<i>Bryophryne cophites</i>	19.8	1																			
<i>Bryophryne hanssaueri</i>	20.8	3.1	4.2																		
<i>Bryophryne nubilosus</i>	19.8	5.2	6.3	2.1																	
<i>Bryophryne phuyuhampatu</i>	18.8	4.2	4.2	3.1	5.2																
<i>Holoaden luederwaldti</i>	18.8	17.7	16.7	14.6	12.5	14.6															
<i>Microkayla chilina</i>	20.8	12.5	12.5	11.5	11.5	9.4	14.6														
<i>Microkayla iatamasi</i>	18.8	11.5	11.5	12.5	12.5	10.4	15.6	3.1													
<i>Microkayla katanitika</i>	18.8	12.5	12.5	9.4	9.4	9.4	11.5	5.2	5.2												
<i>Noblella lochites</i>	12.5	17.7	17.7	15.6	13.5	13.5	12.5	15.6	16.7	15.6											
<i>Psychrophrynella vilcabambensis</i> sp. nov.	16.7	11.5	10.4	12.5	13.5	11.5	18.8	13.5	12.5	13.5	16.7										
<i>Noblella losamigos</i>	16.7	16.7	15.6	15.6	16.7	16.7	14.6	16.7	15.6	15.6	14.6	11.5									
<i>Noblella myrmecoides</i>	16.7	15.6	15.6	15.6	15.6	13.5	16.7	14.6	15.6	13.5	11.5	17.7	21.9								
<i>Noblella pygmaea</i>	15.6	16.7	15.6	16.7	17.7	15.6	15.6	13.5	13.5	14.6	14.6	12.5	8.3	21.9							
<i>Noblella madreselva</i>	15.6	15.6	14.6	16.7	15.6	15.6	14.6	15.6	14.6	16.7	14.6	11.5	8.3	20.8	11.5						
<i>Noblella thiuni</i>	13.5	11.5	12.5	12.5	13.5	12.5	15.6	10.4	9.4	13.5	13.5	9.4	8.3	18.8	7.3	9.4					
<i>Psychrophrynella chirihampatu</i>	11.5	14.6	13.5	15.6	16.7	14.6	14.6	14.6	12.5	13.5	14.6	13.5	9.4	19.8	7.3	8.3	8.3				
<i>Psychrophrynella glauca</i>	15.6	13.5	12.5	14.6	14.6	13.5	18.8	12.5	10.4	11.5	15.6	8.3	12.5	18.8	12.5	11.5	9.4	10.4			
<i>Psychrophrynella usurpator</i>	14.6	17.7	16.7	14.6	13.5	15.6	11.5	15.6	15.6	12.5	12.5	13.5	9.4	19.8	11.5	5.2	11.5	7.3	11.5		
<i>Phrynopus peruanus</i>	20.8	13.5	12.5	13.5	13.5	13.5	16.7	10.4	9.4	9.4	19.8	13.5	17.7	12.5	16.7	17.7	15.6	16.7	13.5	18.8	
<i>Psychrophrynella</i> sp. MUSM27619	16.7	11.5	10.4	12.5	13.5	11.5	18.8	13.5	12.5	13.5	16.7	0	11.5	17.7	12.5	11.5	9.4	13.5	8.3	13.5	13.5



Fig. 3. Morphological details of *Psychrophrynella vilcabambensis* sp. nov. (A) Plantar surface with a red arrow indicating the presence of lateral fringes (MUBI 13484); (B) lateral view of forelimb with white arrows indicating the presence of ulnar tubercles (MUBI 13485).

upper part of pupil. Throat, chest, and anterior part of belly dark brown, fading into light brown posteriorly, with moderate small pale gray flecks and abundant tiny dots of the same color. Ventral parts of limbs brown with moderate small pale gray flecks. Dorsal surfaces of hind limbs with a dark transverse bar. Posterior surfaces of thighs and groin grayish brown; plantar and palmar surfaces brown (Fig. 3).

Coloration in preservative. Similar to coloration in life, but dorsal coloration varies from brown to light brown. The parts that were reddish brown lose the red coloration, and ventral surfaces become more gray than brown.

Variation. Dorsum coloration varies from dark reddish brown to light brown. Flecks on the back are irregular in shape. The dark brown lateral stripe extends from the tip of the snout to the insertion of the forelimb in the holotype, while in the paratypes it reaches the anterior half of the body, and the posterior half has similar coloration as the dorsum. The belly coloration in the female paratype (MUBI 13486) is light brown with abundant tiny pale gray spots. The juvenile paratype (MUBI 13484) has a creamy dorsal midline extending from the tip of the snout to the cloaca; the male holotype and female do not differ in size or general color pattern.

Etymology. The specific epithet, *vilcabambensis*, is given after the name of the mountain range “Cordillera de Vilcabamba” where the species was found.

Distribution, natural history, and threats. *Psychrophrynella vilcabambensis* sp. nov. is known only from elevations of 3,707 m asl in the type locality (Challcha, department of Cusco), near the road between Vilcabamba and Pampaconas (Fig. 4). All specimens were found in high Andean puna (Fig. 5) during the day, under mosses covering the rocks. Sympatric amphibian and reptile species include *Bryophryne flammiventris*, *Nannophryne* sp., and *Proctoporus lacertus*. The type locality suffers from anthropogenic activities, such as farming and livestock production, which might be potential threats to this species. Following the IUCN Red List criteria (IUCN 2019), in the absence of more detailed data concerning population status, extent, and occurrence, we suggest this species be placed in the Data Deficient category of the Red List.

Discussion

The highlands of the Andes of southeastern Peru (departments of Cusco and Puno) are inhabited by 23 species of small, directly developing frogs, with plump

Table 3. Measurements (in mm) of type series of *Psychrophrynella vilcabambensis* **sp. nov.** See text for character abbreviations.

Characters	Female (n = 1)	Male (n = 1)
SVL	16.6	16.5
TL	7.8	7.3
FL	8.5	8.5
HL	4.8	4.7
HW	5.3	5.5
ED	1.8	1.8
TY	0.9	0.9
IOD	2.3	2.2
EW	1.4	1.4
IND	2.0	1.8
E-N	1.3	1.1
TL/SVL	0.5	0.4
FL/SVL	0.5	0.5
HL/SVL	0.3	0.3
HW/SVL	0.3	0.3
HW/HL	1.1	1.2
E-N/ED	0.7	0.6
EW/OID	0.6	0.6
ED/HL	0.4	0.4

bodies and short legs, of the genera *Psychrophrynella*, *Noblella*, *Bryophryne*, *Qosqophryne*, and *Microkayla* (Frost 2020; De la Riva 2020). Likewise, the Andes of southeastern Peru are formed by six cordilleras: Apolobamba, Carabaya, Paucartambo, Urubamba, Vilcabamba, and Vilcanota (ANA 2014; Morales 2010; Lehr and Catenazzi 2008), where each Cordillera is inhabited by more than one species of frog from the subfamily Holoadeninae. Five species (*B. bakersfieldi*, *B. cophites*, *B. hanssaueri*, *B. nubilosus*, and *P. usurpator*) inhabit the Cordillera de Paucartambo (Chaparro et al. 2015; De la Riva et al. 2008; Lehr and Catenazzi 2008, 2009; Lynch 1975), five species (*B. tocra*, *B. willakunka*, *M. boettgeri*, *N. thiuni*, and *P. glauca*) inhabit the Cordillera de Carabaya (Catenazzi and Ttito 2018, 2019; De la Riva et al. 2017; Lehr 2006), five species (*B. phuyuhampatu*, *B. quellokunka*, *B. zonalis*, *P. bagrecito*, and *P. chirihamptu*) inhabit the Cordillera de Vilcanota (Catenazzi and Ttito 2016; Catenazzi et al. 2017; De la Riva et al. 2017; Lehr and Catenazzi 2009; Lynch 1986), three species (*B. abramalagae*, *B. bustamantai*, and *Q. gymnotis*) inhabit the Cordillera de Urubamba (Chaparro et al. 2007; Lehr and Catenazzi 2009, 2010), three species (*P. vilcabambensis* **sp. nov.**, *Q. flammiventris*, and *Q. mancoinea*) inhabit the Cordillera de Vilcabamba (Lehr and Catenazzi 2010, Mamani et al. 2017), and two species (*M. chapi* and *M. chilina*) inhabit the Cordillera de Apolobamba (De la Riva et al. 2017). Eleven of these species were described during the past five years (Frost 2020) from the results of explorations of areas

with difficult access. However, there are still remote and unexplored places which could harbor additional new species or extensions of the ranges of the described species. One example is the study by Catenazzi et al. (2020), which reported three undescribed species in the genus *Psychrophrynella* (*P. sp.P*, *P. sp.R*, and *P. sp.*) and one in the genus *Noblella* (*N. sp.R*). In this context, the diversity of direct-developing Andean frogs that inhabit the high Andes is underestimated, and there is a need for more expeditions to remote locations that lack records, such as much of the Cordillera de Vilcabamba.

Uncorrected *p*-distance analyses of the 16S rRNA sequences show that the new species, *Psychrophrynella vilcabambensis* **sp. nov.**, has a narrow genetic distance from both *P. glauca* (8.3%) and *Noblella thiuni* (9.4%). The new species, *P. glauca*, and *N. thiuni* are found together in the leaf litter at the Thiuni locality, department of Puno, Peru (Catenazzi and Ttito 2018, 2019). The phylogenetic analysis obtained by Catenazzi and Ttito (2019), places *N. thiuni* as a sister group of the species of *Psychrophrynella* and *Noblella* in southern Peru. Therefore, the fact that *P. vilcabambensis* **sp. nov.** and *N. thiuni* have a narrow genetic distance, despite having a geographical distance of 285 km in a straight line, provides further evidence for the phylogenetic and taxonomic uncertainty between the species of the genera *Psychrophrynella* and *Noblella* (Catenazzi and Ttito 2018, 2019; De la Riva et al. 2008, 2017).

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Literature Cited

ANA. 2014. *Inventario de Glaciares del Perú (2da Actualización)*. Unidad de glaciología y recursos hídricos (UGRH), Huaraz, Peru. 198 p.

Canedo C, Haddad CF. 2012. Phylogenetic relationships within anuran clade Terrarana, with emphasis on the placement of Brazilian Atlantic rainforest frog genus *Ischnocnema* (Anura: Brachycephalidae). *Molecular Phylogenetics and Evolution* 65(2): 610–620.

Catenazzi A, Ttito A. 2016. A new species of *Psychrophrynella* (Amphibia, Anura, Craugastoridae) from the humid montane forests of Cusco, eastern

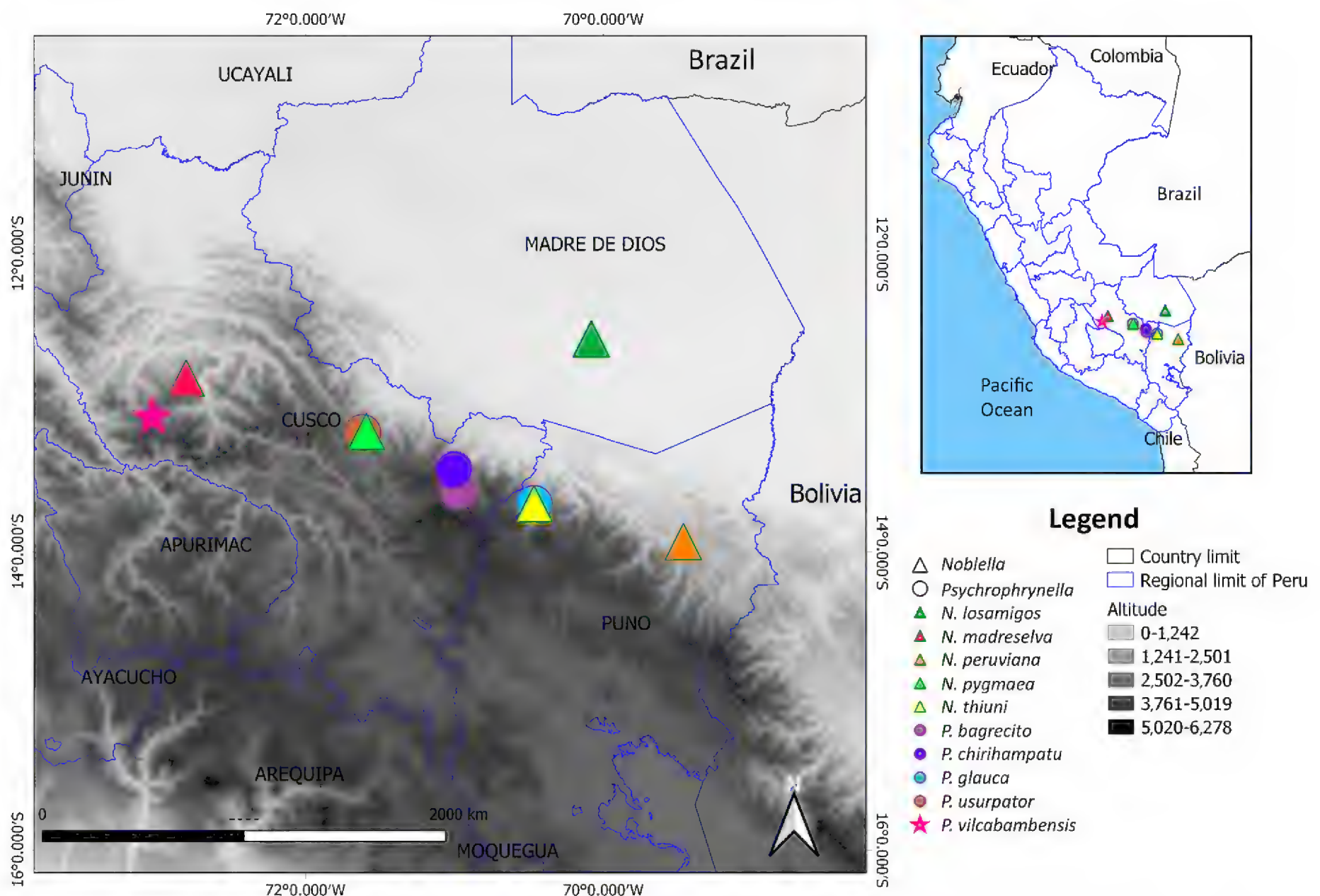


Fig. 4. Map of Peru indicating the type localities of species of *Psychrophrynella* and *Noblella* from the southeastern region of the country.

slopes of the Peruvian Andes. *PeerJ* 4: 1–22.

Catenazzi A, Ttito A. 2018. *Psychrophrynella glauca* sp. n., a new species of terrestrial-breeding frogs (Amphibia, Anura, Strabomantidae) from the montane forests of the Amazonian Andes of Puno, Peru. *PeerJ* 6: 1–18.

Catenazzi, A, Ttito A. 2019. *Noblella thiuni* sp. n., a new (singleton) species of minute terrestrial-breeding frog (Amphibia, Anura, Strabomantidae) from the montane forest of the Amazonian Andes of Puno, Peru. *PeerJ* 7: 1–18.

Catenazzi A, Uscapi V, von May R. 2015. A new species of *Noblella* (Amphibia, Anura, Craugastoridae) from the humid montane forests of Cusco, Peru. *ZooKeys* 516: 71–84.

Catenazzi A, Ttito A, Diaz MI, Shepack A. 2017. *Bryophryne phuyuhampatu* sp. n., a new species of Cusco Andes frog from the cloud forest of the eastern slopes of the Peruvian Andes (Amphibia, Anura, Craugastoridae). *ZooKeys* 685: 65–81.

Catenazzi A, Mamani L, Lehr E, von May R. 2020. A new genus of terrestrial-breeding frogs (Holoadeninae, Strabomantidae, Terrarana) from Southern Peru. *Diversity* 12(5): 1–17.

Chaparro JC, De la Riva I, Padial JM, Ochoa JA, Lehr E. 2007. A new species of *Phrynosoma* from Departamento Cusco, southern Peru (Anura: Brachycephalidae). *Zootaxa* 1618: 61–68.



Fig. 5. View of the type locality of *Psychrophrynella vilcabambensis* sp. nov.

Chaparro JC, Padial JM, Gutiérrez RC, De la Riva I. 2015. A new species of Andean frog of the genus *Bryophryne* from southern Peru (Anura: Craugastoridae) and its phylogenetic position, with notes on the diversity of the genus. *Zootaxa* 3994: 94–108.

De la Riva I. 2020. Unexpected beta-diversity radiations in highland clades of Andean terranean frogs. Pp. 741–764 In: *Neotropical Diversification: Patterns and Processes. Fascinating Life Sciences*. Editors, Rull V, Carnaval AC. Springer, Cham, Switzerland. 820 p.

De la Riva I, Aparicio J. 2016. Three new Bolivian species of *Psychrophrynella* (Anura: Craugastoridae), and

- comments on the amphibian fauna of the Cordillera de Apolobamba. *Salamandra* 52: 283–292.
- De la Riva I, Burrowes PA. 2014. A new species of *Psychrophrynella* (Anura: Craugastoridae) from the Cordillera Real, Department La Paz, Bolivia. *Zootaxa* 3887: 459–470.
- De la Riva I, Chaparro JC, Padial JM. 2008. A new, long-standing misidentified species of *Psychrophrynella* Hedges, Duellman, and Heinicke from Departamento Cusco, Peru (Anura: Strabomantidae). *Zootaxa* 1823: 42–50.
- De la Riva I, Chaparro JC, Castroviejo-Fisher S, Padial JM. 2017. Underestimated anuran radiations in the high Andes: five new species and a new genus of Holoadeninae, and their phylogenetic relationships (Anura: Craugastoridae). *Zoological Journal of the Linnean Society* 182(1): 129–172.
- Duellman WE, Lehr E. 2009. *Terrestrial-Breeding Frogs (Strabomantidae) in Peru*. Natur und Tier Verlag, Münster, Germany and Ulrich Manthey, Berlin, Germany. 386 p.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1,792–1,797.
- Frost DR. 2020. Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database. Available: <https://amphibiansoftheworld.amnh.org/index.php> [Accessed: 29 August 2020].
- Hedges SB, Duellman WE, Heinicke MP. 2008. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1–182.
- Heinicke MP, Lemmon AR, Lemmon EM, McGrath K, Hedges SB. 2018. Phylogenomic support for evolutionary relationships of New World direct-developing frogs (Anura: Terraranae). *Molecular Phylogenetics and Evolution* 118: 145–155.
- IUCN Standards and Petitions Committee. 2019. *Guides for using the IUCN Red List Categories and Criteria. Version 14*. Prepared by the Standards and Petitions Committee. IUCN, Gland, Switzerland. 133 p.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1,547–1,549.
- Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30(22): 3,276–3,278.
- Lehr E. 2006. Taxonomic status of some species of Peruvian *Phrynosoma* (Anura: Leptodactylidae), with the description of a new species from the Andes of southern Peru. *Herpetologica* 62: 331–347.
- Lehr E, Catenazzi A. 2008. A new species of *Bryophryne* (Anura: Strabomantidae) from southern Peru. *Zootaxa* 1784(1): 1–10.
- Lehr E, Catenazzi A. 2009a. A new species of minute *Noblella* (Anura: Strabomantidae) from southern Peru: the smallest frog of the Andes. *Copeia* 2009(1): 148–156.
- Lehr E, Catenazzi A. 2009b. Three new species of *Bryophryne* (Anura: Strabomantidae) from the region of Cusco, Peru. *South American Journal of Herpetology* 4(2): 125–138.
- Lehr E, Catenazzi A. 2010. Two new species of *Bryophryne* (Anura: Strabomantidae) from high elevations in southern Peru (Region of Cusco). *Herpetologica* 66(3): 308–319.
- Lynch JD. 1975. A review of the Andean leptodactylid frog genus *Phrynosoma*. *Occasional Papers of the Museum of Natural History, University of Kansas* 35: 1–51.
- Lynch JD. 1986. New species of minute leptodactylid frogs from the Andes of Ecuador and Peru. *Journal of Herpetology* 20(3): 423–431.
- Lyra ML, Haddad CF, de Azeredo-Espin AML. 2017. Meeting the challenge of DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers. *Molecular Ecology Resources* 17(5): 966–980.
- Mamani L, Catenazzi A, Tito A, Mallqui S, Chaparro JC. 2017. A new species of *Bryophryne* (Anura: Strabomantidae) from the Cordillera de Vilcabamba, southeastern Peruvian Andes. *Phyllomedusa* 16: 129–141.
- Morales C. 2001. *Las Cordilleras del Perú*. Banco Central de Reserva del Perú. Consejo Editorial USMP, Lima, Peru. 201 p.
- Noble GK. 1921. Five new species of Salientia from South America. *American Museum Novitates* 29: 1–7.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The Simple Fool's Guide to PCR. Version 2.0*. Privately published document compiled by S. Palumbi, Department of Zoology, University of Hawaii, Honolulu, Hawaii, USA. 47 p.
- Reyes-Puig JP, Reyes-Puig C, Ron SR, Ortega JA, Guayasamin JM, Goodrum M, Recalde F, Vieira J, Koch C, Yáñez-Muñoz MH. 2019. A new species of terrestrial frog of the genus *Noblella* Barbour, 1930 (Amphibia: Strabomantidae) from the Llanganates-Sangay Ecological Corridor, Tungurahua, Ecuador. *PeerJ* 7: 1–26.
- Santa Cruz R, von May R, Catenazzi A, Whitcher C, López Tejada E, Rabosky DL. 2019. A new species of terrestrial-breeding frog (Amphibia, Strabomantidae, *Noblella*) from the Upper Madre De Dios watershed, Amazonian Andes and lowlands of southern Peru. *Diversity* 11(9): 1–20.
- von May R, Catenazzi A, Corl A, Santa-Cruz R, Carnaval AC, Moritz C. 2017. Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution* 7(9): 3,257–3,267.
- von May R, Lehr E, Rabosky DL. 2018. Evolutionary radiation of earless frogs in the Andes: molecular phylogenetics and habitat shifts in high-elevation terrestrial breeding frogs. *PeerJ* 6: 1–27.



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Appendix 1. Specimens examined.

Psychrophrynella bagrecito ($n = 4$): **PERU. Cusco:** Quispicanchi: Camanti: Iskaybamba: MUBI 5255–58.

Psychrophrynella chirihampatu ($n = 12$): **PERU. Cusco:** Paucartambo: Área de Conservación Privada (ACP) Ukumari Llaqta: MUBI 14656, MUBI 14658, MUBI 14661–14662, MUBI 14664, MUBI 14666–72 (paratypes).

Psychrophrynella glauca ($n = 1$): **PERU. Puno:** Thiuni: Ollachea: MUBI 16323 (paratype).

Psychrophrynella usurpator ($n = 3$): **PERU. Cusco:** Paucartambo: Acjanacu: MUBI 4642–43 (paratypes).



Evidence for cryptic diversity in the Neotropical water snake, *Helicops angulatus* (Linnaeus, 1758) (Dipsadidae, Hydropsini), with comments on its ecology, facultative reproductive mode, and conservation

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Abstract.—The neotropical aquatic snake genus *Helicops* contains 19 species, some of which are oviparous, while others are viviparous. Using phylogenetic and morphological relationships, *H. angulatus* from the island of Trinidad (West Indies) is compared to other mainland populations. We recover *H. angulatus* as paraphyletic, suggesting evidence of cryptic diversity within the species, and remove *Helicops cyclops* Cope, 1868 from the synonymy of *Helicops angulatus* (Linnaeus) based on morphology; thus, increasing the number of *Helicops* species to 20. Previous reports suggest some populations of the widespread *Helicops angulatus* are oviparous. In contrast, other populations have been reported as viviparous, and the conflicting reports are discussed based upon recent literature on the evolution of viviparity. Anecdotal evidence suggests Trinidad *Helicops angulatus* are facultatively viviparous. The importance of conserving this unusual population, and its associated aquatic habitats, are discussed.

Keywords. Caribbean, neotropics, ovoviviparous, Reptilia, Squamata, viviparous

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Introduction

Most of the 3,700 species of snakes are terrestrial, but a few hundred have become aquatic or semi-aquatic in freshwater and marine environments (Murphy 2012). Two-thirds of 33 family and subfamily level clades contain aquatic species (Murphy 2012). Some clades contain only a single extant species that can be considered semi-aquatic or aquatic (e.g., Viperidae), while others include dozens of species that have radiated into freshwater habitats (e.g., Homalopsidae and the Natricidae). Current knowledge suggests the most diverse aquatic snake communities occur in southeast Asia, but a significant number of radiations into freshwater are present in cis-Andean South America. At least 50 species in 12 genera

can be considered semi-aquatic or aquatic among the species which inhabit the Amazon Basin and adjacent areas (Murphy 2012).

About half of those neotropical species are in the Dipsadidae lineage Hydropsini (Dowling 1975), a clade of 23 freshwater and brackish water snakes in three genera: *Helicops* Wagler, 1828 (19 species), *Hydrops* Wagler, 1830 (three species), and *Pseudoeryx* Fitzinger, 1826 (two species). The relationship of *Helicops*, *Hydrops*, and *Pseudoeryx* was suggested by Roze (1957), while Dowling (1975) provided a name for the clade, and Zaher (1999) hypothesized the three genera formed a clade belonging to the Xenodontinae. Molecular evidence supporting the Hydropsini first came from Vidal et al. (2000), when they recovered *Hydrops* and *Pseudoeryx* as the sister

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to *Helicops*. Kelly et al. (2003) also found Hydropsini to be monophyletic. Since that time, Grazziotin et al. (2012) recovered strong support for the clade with its sister, the terrestrial, Mexican lizard-eating snake *Manolepis*. The Hydropsini was again found to be monophyletic by Zaher et al. (2018). Vidal et al. (2010) also confirmed the Hydropsini clade and presented molecular evidence that *Helicops angulatus* is paraphyletic.

Di Pietro et al. (2014a,b) described what appeared to be synapomorphies in the skull and upper respiratory systems that supported the monophyly of the Hydropsini. They (Di Pietro et al. 2014a) found two nasal features that deviate from the pattern of nasal cartilages known in other alethinophidian and caenophidian snakes: i.e., a cartilaginous connection between the lamina transversalis anterior and the concha of the Jacobson's organ, and a small rostral projection of the planum anteorbital, which probably represents a reduced anterior maxillary process. They (Di Pietro et al. 2014b) later found the unpaired foramen on the parabasisphenoid with an anterior position to be the only skull characteristic shared by all Hydropsini genera.

Viviparity has also been suggested as a synapomorphy for the Hydropsini (Zaher et al. 2009); however, contrary to previous speculation, *Hydrops* is oviparous, and *Pseudoeryx plicatilis* is now known to lay eggs with the female attending the nest (Braz et al. 2016). However, within *Helicops*, some species are oviparous, while others are viviparous (Scartozzoni 2009; Braz et al. 2016).

Rossman (1973) reviewed the early conflicting evidence that suggested *Helicops angulatus* was viviparous. He reported that a female from Leticia (Colombia) laid two atypical eggs with the well-developed embryos visible through the shells. He incubated the eggs, and they hatched 16 and 17 days later. Speculating on the reproductive mode of *H. angulatus*, he wrote, "... there is a remote possibility that even this species may be facultatively ovoviviparous." Continuing, he discussed the advantage of an aquatic snake being viviparous if egg-laying sites were in short supply at times of severe flooding.

Oviparity and viviparity are used to describe the reproductive modes in squamates. The term "ovoviviparity" was used until the mid-20th century with the intention of defining an intermediate strategy between viviparous and oviparous reproductive modes. However, this word was eliminated because of ambiguity caused by the variety of interpretations of its meaning (Blackburn 1994, 2000, 2006). A bipartite classification was proposed by Blackburn (2000) that distinguishes between two parameters: (1) the source of nutrition for embryonic development (lecithotrophy and matrotrophy, as two extremes of a continuum where lecithotrophy indicates the embryo depends entirely on yolk, and matrotrophy indicates the embryo obtains most of its nutrition via a placenta); and (2) the packaging of the embryo (an eggshell or membrane containing the young). While the majority of Squamata are clearly either oviparous or

viviparous, there are reports of some species which use both reproductive modes.

In a review of oviparity and viviparity in squamates, Tinkle and Gibbons (1977) listed 12 species (four lizards and eight snakes) reported in the literature to have bimodal or facultative reproduction. That is, 12 species that use both oviparity and viviparity, including eight snakes (*Typhlops diardi*, *Boa constrictor*, *Python regius*, *Diadophis punctata*, *Xenodermus javanicus*, *Cacophis kreffti*, *Echis carinatus*, and *Trimeresurus okinavensis*). They discuss each of these literature reports and the implications of females retaining embryos in their bodies until they are well developed before secreting an eggshell around the embryo and depositing the eggs in a nest.

Regarding *Helicops*, Rossman (1984) provides an account of *Helicops angulatus* (LSUMZ 27337) from Puerto Maldonado (Peru) collected by Richard Thomas. When Thomas preserved the female, he removed seven full-term young (LSUMZ 27340–46). No eggshells or yolk were present, suggesting that had this female *H. angulatus* carried the embryos to full term, she would have functioned as a viviparous, as opposed to an oviparous, species. In discussing Trinidad *Helicops*, Boos (2001) stated that Rodriguez saw a female giving live birth, citing an unpublished manuscript that was missing pages. Ford and Ford (2002) studied *Helicops angulatus* in Trinidad, and reported two females laid clutches of 11 and 18 eggs in February that required 45 and 39 days of incubation, respectively.

The distribution of *Helicops angulatus* is given by Uetz et al. (2020) as "Venezuela (Amazonas, Apure, Bolívar, Monagas, Delta Amacuro, Sucre, Portuguesa, Anzoátegui, Guárico, Cojedes), Colombia, Brazil (Pará, Rondônia, Goiás, Mato Grosso, Sergipe, S. Ceará, Acre, Bahia, Piauí, Paraíba, etc.), Bolivia, Peru, Trinidad, Ecuador, French Guiana, Guyana." Many authors writing about the distribution of *H. angulatus* suggest it is widespread in northern South America (Cunha and Nacimiento 1978; Cisneros-Heredia 2006; Roberto et al. 2009; Starace 2013; Cole et al. 2013; Nogueira et al. 2019).

Given the relatively broad distribution of *Helicops angulatus* and the possible bimodal reproduction of this snake, the Trinidad and Venezuelan populations merit further investigation. The Trinidad and Venezuelan *Helicops* is not likely to be an endemic cryptic taxon to the region. *Helicops angulatus* is a mostly lowland aquatic snake present in the Orinoco Delta, the Llanos, and possibly in the low wetlands of the Guiana Shield. Charles (2013) reported finding a juvenile *Helicops* that had washed up on the South coast of Trinidad with a mat of vegetation, suggesting a flood event transported the snake the short distance from the Orinoco Delta to Trinidad.

Here, through phylogenetic analyses, we present evidence that confirms *H. angulatus* is paraphyletic. We compared the morphological data from the literature and

photographs for five type specimens with names that had been placed in the synonymy of *Helicops angulatus*; and based on that information, we reinstate *Helicops cyclops* Cope, 1868 as a valid species. We also further investigate the distribution, ecology, conservation, and the anecdotal story of a Trinidad *H. angulatus* giving live birth.

Materials and Methods

Molecular methods. DNA was extracted from tissue samples, and target gene fragments were amplified by Polymerase Chain Reaction using the DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) following the manufacturers' instructions. Primers and specimens sequenced and the GenBank accessions for all species are reported in Supplemental Tables S1 and S2. Portions of the mitochondrial small and large ribosomal subunits (12S rDNA, 16S rDNA, respectively), cytochrome *b* (*cytb*), and the nuclear oocyte maturation factor (*c-mos*) were amplified. These gene fragments are highly informative in interspecific and intraspecific studies on snakes, including colubroids (e.g., Daza et al. 2009).

Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous base-calls in Sequencher v4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The lengths of the sequences were: 12S rDNA, 342 base pairs (bp); 16S rDNA, 436 bp; *cytb*, 1,060 bp; *c-mos*, 492 bp, although not all individuals had the exact same length in some alignments. *Cytb* and *c-mos* were translated to amino acids to find the presence of stop codons. Following Moraes Da Silva et al. (2019), the analysis included all genera that were sister to *Helicops* and *Pseudoeryx plicatilis* and *Hydrops triangularis* were used as outgroups. Sequences were aligned in Seaview v4.2.11 (Gouy et al. 2010) under MAFFT settings (Katoh et al. 2002). The 12S and 16S rDNA, and *c-mos* sequences were concatenated, resulting in a 1,271 bp alignment. The *cytb* sequences were used to assess genetic differentiation within the Trinidad specimens. Because of the lack of *cytb* for *Helicops*, this gene was not included in the concatenated alignment.

Phylogenetic analyses were performed using the Bayesian Inference and Maximum Likelihood methods. MrBayes v3.2 (Ronquist and Huelsenbeck 2003) was used to construct the Bayesian Inference tree under the best-fitting substitution model for each gene partition. ML searches were conducted in RAxML v7.0.4 (Silvestro and Michalak 2010), using partition data sets under default settings, and support was assessed by using 1,000 bootstrapped replicates. The most appropriate substitution model was implemented for each gene fragment as determined by the Bayesian Information Criterion in PartitionFinder v2 (Lanfear et al. 2012) to choose the optimal partitioning strategy for both phylogenetic analyses. Default priors and Markov chain settings were used, and searches were performed with random starting

trees. Each run consisted of four chains of 20,000,000 generations, sampled every 2,000 generations. Posterior distributions of parameter estimates were visually inspected in Tracer. All analyses were performed through the CIPRES platform (Miller et al. 2010).

Distributional analysis methods. The Vertnet and GBIF databases were searched for mappable specimens of *Helicops angulatus*. Additional specimens examined from Trinidad and Venezuela that were not represented in the on-line databases, and specimens reported in Appendix B of Braz et al. (2016), were added. All localities were plotted in ARCVIEW (Fig. 1). Additional localities from the map used in Nogueira et al. (2019) were added to the map using Photoshop, and are indicated by the smallest black markers in Fig. 1. The ARCVIEW layers used for the map were: The World Topographic Map, The World Hydro Reference Overlay Map, and Freshwater Ecoregions of the World.

Morphological methods. Traditional scale count data were collected for 37 specimens from Trinidad and Venezuela; and an additional eight specimens from Brazil, Guyana, and Peru were examined. Sex was determined by tail shape, tail length, and visual inspection of the hemipenes. Dorsal scales were counted on the neck at about the 10th ventral, midbody, and about 10 ventral scales anterior to the vent, and they were all counted on the diagonal. Ventral counts, subcaudal counts, and tail/SVL (snout-vent length) ratios were analyzed for sexual dimorphism. Scale counts and scale measurements were taken under a dissection microscope on small specimens. Scale measurements were taken with a metric ruler and dial calipers. Snake sizes are given in millimeters. Scale counts separated by a dash (–) represent a range taken from different individuals; while those separated by a slash (/) represent data taken from a single individual in the left/right order. *Helicops angulatus* have keeled subcaudal scales; and since this character is unfamiliar to many herpetologists and is easily overlooked, it is illustrated in Fig. 2.

Specimens examined: Brazil (*n* = 4): ANSP 5131–2; CAS-SUR 7436, CAS 49324; Guyana (*n* = 2): FMNH 26647, FMNH 170765; Peru (*n* = 2): FMNH 81527, CAS 8720; Trinidad (*n* = 18): CAS 231757, 231758–60; FMNH 251219; UWIZM 2010.27.2 (*n* = 3), 2010.12.93, 2011.20.30 (*n* = 2), 2013.16.1 (*n* = 7); Venezuela (*n* = 16) MHNLS 1429, 1439, 8444, 9093, 9884, 10953, 11786, 17731, 12082, 13137, 14100, 14426, 15885, 17544–45, 18404.

Results

Molecular results. No stop codons were found in the *cytb* and *c-mos* alignments. The best-fitting models and partitions were partition 1 (TRN+G 12s+16S rDNA), partition 2 (JC+I *c-mos* 1st+2nd codon positions), and



Fig. 1. The distribution of *Helicops angulatus* in the Neotropics. Locality data is from the VertNet and GBIF databases, as well as the literature. Diamonds (green oviparous, yellow viviparous): specimens reported in Appendix B of Braz et al. (2016); red stars represent localities where *Helicops* was sampled for DNA; small black markers: localities from *Helicops angulatus* map in Nogueira et al. (2019). As currently defined *Helicops angulatus* occurs in Freshwater Ecoregions: 301 North Andean Pacific Slopes, Rio Atrato; 302 Magdalena, Sinu; 304 South America Caribbean Drainages, Trinidad; 307 Orinoco Llanos; 308 Orinoco Guiana Shield; 311 Guianas; 313 Western Amazon Piedmont; 317 Ucayali, Urubamba Piedmont; 318 Mamore, Madre de Dios Piedmont; 319 Guapore, Itenez; 320 Tapajos, Juruena; 321 Madeira Brazilian Shield; 323 Amazonas Estuary and Coastal Drainages; 324 Tocantins, Araguaia; 325 Parnaiba; and 328 Northeastern Mata Atlantica.

partition 3 (F81+I cmos 3rd codon positions). All six *H. angulatus* from Trinidad recovered the same haplotype for all genes. The two GenBank *H. angulatus* are the sister clade to *H. gomesi*, as shown previously (Moraes Da Silva et al. 2019). However, the inclusion of Trinidad's *H. angulatus* results in paraphyly of the species; the island taxon is ancestral to mainland *Helicops* + *H. gomesi* (Fig. 3). All clades were recovered with high bootstrap and posterior probabilities.

The morphological results (Table 1) suggest that animals from Trinidad and Paria, Venezuela, are the same species and are like some other mainland populations in that they have nearly identical meristic traits. Dorsal scale rows, ventral counts, and subcaudal counts are similar for specimens examined and when compared to literature accounts. Boulenger (1893) may have first reported the

keeled subcaudals, which are difficult to detect because they are lateral (Rossman 1973). The Trinidad and Venezuela populations also have the first dorsal scale row lacking keels.

Distribution and ecology. The distribution of *Helicops angulatus* is shown in Fig. 1, and it extends well outside the Amazon Basin. However, there are some records in Colombia that need attention; one of which is a specimen (FLMNH 57235) from the Atlántico Province, which has a locality within 15 km of the coast, west of the Maracaibo Basin. A second is the specimen collected in the Cordillera Central (Sonsón, Antioquia) at 2,300 m (ILS no. 92) and mentioned in Pérez-Santos and Moreno (1988). However, the identification of this specimen requires confirmation. A third specimen (ICN MHN Rep



Fig. 2. Photo showing that *Helicops angulatus* has keeled subcaudal scales, a trait that is easily overlooked because the keels are lateral. Photo by Renoir J. Auguste.

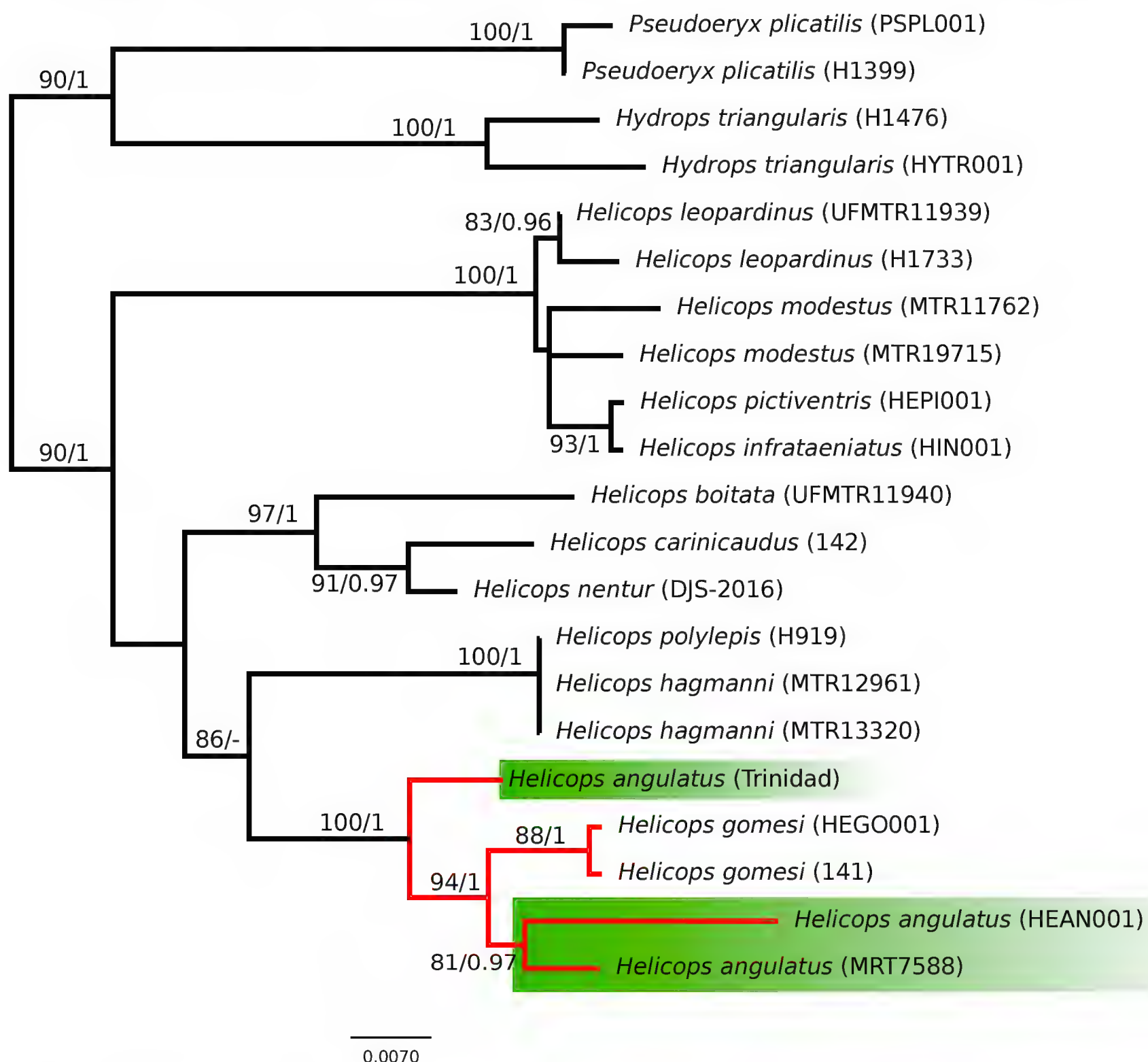


Fig. 3. Best Maximum Likelihood tree based on the data set of concatenated 12S and 16S rDNA, and c-mos sequences. The red clade depicts the *Helicops angulatus* group. On the left and right sides of a slash (/) are values indicated at nodes for Maximum Likelihood bootstraps (> 75%) and Bayesian Posterior probability values (> 95%), respectively. Green clades represent the paraphyly of *Helicops angulatus*. The name *Helicops pictiventris* is currently a junior synonym of *H. infrataeniatus*, but it appears in the tree exactly as the pertinent sequences appear in the GenBank dataset.

Table 1. Morphological comparisons of the type specimens of species that have been synonymized under *Helicops angulatus* to the Trinidad/Venezuela population. The Trinidad and Venezuela animals are sexually dimorphic for body size. Six adult males have SVL 351–560 mm (\bar{x} = 421.6, SD = 65.13), tails 130–219 (\bar{x} = 177.3, SD = 30.85), and tail/SVL ratios 0.37–0.48 (\bar{x} = 0.42, SD = 0.046). Eight males had ventral counts 110–118 (\bar{x} = 113.5, SD = 3.27) and subcaudal counts 70–83 (\bar{x} = 75.12, SD = 4.42). Ten adult females have SVL 337–838 (\bar{x} = 575.42, SD = 142.06), tails 98–224 (\bar{x} = 159.80, SD = 36.04), and tail/SVL ratios 0.22–0.36 (\bar{x} = 0.29, SD = 0.04). Female ventral counts for 21 individuals ranged from 113–123 (\bar{x} = 118.48, SD = 2.48); subcaudal counts for 18 females ranged from 59–77 (\bar{x} = 65.33, SD = 5.04); tail/SVL ratios for ten females ranged from 0.22–0.36 (\bar{x} = 0.29, SD = 0.04). nd = no data.

Locality	<i>alidars</i> Linnaeus		<i>angulatus</i> Linnaeus		<i>surinamensis</i> Shaw		<i>asper</i> Wagler		<i>fumigatus</i> Cope		<i>cyclops</i> Cope	
	?		Suriname		Suriname		Brazil		Suriname		Brazil	Trinidad/ Eastern Venezuela
Ventral scales	121		120		nd		123		nd		124	109–121
Subcaudal scales	58		61		nd		82		nd		89	59–83
Dorsal pattern extends to venter	nd		yes		yes		yes		no		yes	yes
t/SVL	nd		0.36		nd		0.479		nd		nd	0.37–0.48
Dorsal scale rows	21		19		19		19		19		19	19
Keels on first scale row	nd		no		nd		nd		no		yes	no
Keels on subcaudals	nd		nd		nd		nd		yes		yes	yes
Temporal formula	nd		1+2		nd		2+3		1+2		2+3	1+2
Dorsal transverse bands on midline	nd		36		nd		46		nd		nd	32–43

10735) from Department of Tolima, west of Bogota, was listed in the GIBF database as being from 680 m, but the coordinates given in Google Earth suggest the elevation is quite different, closer to 2,600 m. *Helicops angulatus* is a species restricted in Colombia to the Amazon and Orinoco basin, but the first specimen mentioned above (FLMNH 57235) is almost surely *H. danieli* (Rossman 2002); while the other two individuals most likely represent records with erroneous collection data, and for this reason, they cannot be considered within the geographic distribution of this species. Another specimen (LACM 58898) from near Lima, Peru on the West side of the Andes is likely the result of human transport, as the coordinates suggest it is from a highly urbanized area.

In Trinidad and Venezuela, 37 specimens were found from 26 localities, ranging from sea level in the coastal regions (Trinidad: Caroni Swamp, Nariva Swamp; Venezuela: Orinoco Delta region, Llanos), up to 940 m in forested streams of the Venezuelan Guayana. *Helicops angulatus* occurs in all freshwater systems in Trinidad, while it occurs throughout the Orinoco Basin in Venezuela. Figure 1 documents its presence in 16 freshwater ecoregions.

This species is abundant in slow-moving or stagnant water bodies, such as coastal lagoons, ponds, swamps, grasslands, flooded riparian forest, and mangroves, where the water may be clear, turbid due to high sediment loads, or black with high concentrations of tannic acid (Sioli 1975). It also occurs in bodies of water modified by humans (Ford and Ford 2002; Lasso et al. 2014; Mohammed et al. 2014). Accordingly, Ford and Ford (2002) found it to be abundant in a flooded watermelon field, from which they collected 117 specimens in five days.

Three specimens (MHNLS 17731, 13137, 10943) were collected in the lower Orinoco basin, as well as in an estuary and on two fluvial islands. These localities are on the northern edge of the range and are likely to be influenced by tides. These specimens were captured between March and June, when water flow and precipitation decrease salinity levels (Novoa 2000; Lasso and Sánchez-Duarte 2011). However, the occurrence of *H. angulatus* in mangroves is evidence that it is tolerant of some degree of salinity.

Morphological and systematic results. Linnaeus (1758: 217) described *Coluber angulatus* based on the type NRM 17 (Fig. 4) said to be from Asia (in error). The type specimen has 120 ventral scales and 60 subcaudal scales, and came from King Adolf Fredrik’s collection at Ulricsdal Castle, Sweden. After it was examined by Linnaeus, it was transferred to KVA/NRM (Royal Swedish Academy of Science/Swedish Museum of Natural History) in 1801 (Anderson 1899).

Linnaeus appears to have described *C. angulatus* a second time as *Coluber alidras* based upon NRM 18, which originated in the collection of Charles De Geer (= Mus. De Geer) and gave the type locality as “Indiis.”



Fig. 4. NRM 17, the holotype for *Helicops angulatus*. Photo courtesy of NRM.

Andersson (1899: 34) examined *Coluber alidras* and commented that it is a “...completely discolored specimen of *Helicops angulatus*.” However, it differed from *C. angulatus* by having 21 rows of scales on the thickest part of the body instead of 19 (in *Coluber angulatus*). Thus, Andersson considered Linnaeus’s *Coluber alidras* a synonym of *Coluber angulatus*. The *C. alidras* specimen had a total length of 720 mm and a 165 mm tail, 121 ventral scales, and 58 subcaudal scales. He (Andersson 1899) added a footnote stating that the tail was somewhat mutilated. NRM 18 contained the remains of the fish *Cichlasoma bimaculatum*, which has a South American distribution that includes the Orinoco River basin, in the Caroni in River Venezuela; Guianas, from the Essequibo River to the Sinnamary River; and the Amazon River

basin, in the upper Branco River basin (Froese and Garilao 2019).

Shaw (1802: 460) described *Coluber surinamensis*, stating that it was thought to be from Suriname and listed the holotype as a drawing (Sebae, 1735, Vol. 2, Pl. 59, Fig. 2) [Fig. 5]. The specimen used for that illustration survives to the present day. Bauer and Wahlgren (2013) examined some of the snake specimens from the 18th century Linck family collection that are in the Naturalienkabinett Waldenburg in Saxony, Germany. Specimens in that collection served as types of species described by Linnaeus and Blasius Merrem, and are thus of taxonomic importance. For example, specimen 502 is the basis for the illustration holotype of Shaw’s *Coluber surinamensis*.



Fig. 5. Illustration of the holotype of *Coluber surinamensis* Shaw. From Sebae (1735, Vol. 2, pl. 59, Fig. 2).

Eighteenth century collectors sent many animals (alive and preserved) from Suriname to the Netherlands. The preserved specimens found their way into private collections and Linnaeus undoubtedly saw many Suriname specimens because the collectors C.G. Dahlberg and D. Rolander were sending Suriname specimens to Sweden (Husson 1978). Thomas (1911) wrote that it would not be unjustified to regard all South American animals in *Seba's Thesaurus* as originating in Suriname. Therefore, we consider it likely that NRM 17, NRM 18, and the Linck family collection specimen 502 used in Seba's drawing and Shaw's description all originated in Suriname.

Wagler (1824: 37) described *Natrix aspera* which is now represented by the lectotype (ZSM 1528/0). Hoogmoed and Gruber (1983) commented that the original Spix collection contained adults and juveniles, and gave scale counts for two specimens (123 and 118 ventral scales and 82 and 100 subcaudals, respectively). However, they found that only one Brazilian specimen collected by Spix was still present in the Munich collection and selected it as the lectotype of *Natrix aspera* Wagler. The lectotype (Fig. 6), is a female which has 123 ventrals, a divided cloacal plate, 82 subcaudals, and dorsal scales in 19-19-17 rows. The SVL is 690 mm, the tail length is 331 mm ($t/SVL = 0.479$), the head length is 38.8 mm, and it has maxillary teeth (± 14). The specimen agrees well with Wagler's description except for the pattern. Subsequently, Wagler (1830: 171) erected the genus

Helicops for *Coluber angulatus* and used the combination *Helicops angulatus*.

Cope (1868: 308) described *Helicops fumigatus* based on the holotype: ANSP 5132 from Suriname, stating that it has keeled scales that are in 19 rows and provided no other information on scale characters. However, he described this snake on the basis of the ventral pattern, writing, "Belly with a broad brownish gray band from throat to vent, the ends of the gastrosteges yellow, forming two bands; a median longitudinal brown line on the tail." We have not seen any *Helicops angulatus* with this ventral pattern nor a mid-ventral stripe on the tail (Fig. 7).

In the same paper, immediately following the *H. fumigatus* description, Cope (1868: 309) described *Helicops cyclops* based on the holotype ANSP 5131 from Bahia, Brazil. The specimen has 19 dorsal scale rows, 124 ventral scales, and 89 subcaudals, and it has 26 dark brown transverse bands. Boulenger (1893: 279) placed both of Cope's species into the synonymy of *Helicops angulatus* without comment.

Helicops cyclops has a remarkably short head and more ventral scales than have been reported for *Helicops angulatus*. Its subcaudal scales are keeled. It also has chin shields that are short and plate-like, and dorsal transverse bands which extend around the body and across the ventral surface. There is also a distinctive band between the eyes, a trait not seen in members of the *Helicops angulatus* group. Dorsal head plates are also imbricate,

more so than those seen in members of the *H. angulatus* group. Based on this morphology, we remove *Helicops cyclops* Cope from the synonymy of *H. angulatus*.

***Helicops cyclops* Cope (1868)**

Fig. 8

Helicops cyclops Cope 1868: 309. Holotype ANSP 5133, Type locality Bahia, Brazil.

Helicops angulatus – Boulenger 1893, 2: 287.

Cope's description of this species is as follows:

Helicops cyclops Cope, sp. nov.

Scales in nineteen rows, strongly keeled everywhere, including the first row. Two pairs genials; occipitals short and wide, long as frontal. Head exceedingly short, mouth wide as the length of the rounded lip margin. Superior labials eight, fourth scarcely entering orbit by its upper corner (by its whole extremity in *H. angulatus*), the fifth, sixth and seventh narrow and high. Prefrontals broad as long (much broader than long in *H. angulatus*). Orbitals 1–2, nearly meeting below orbit. Temporals 2 | 1 | 3 (1 | 1 | 3 in *H. angulatus*). Loreal plate wide as high, (higher than wide in *H. angulatus*). Tail 0.33 total length, slender (less than 0.25, *H. angulatus*). Gastrasteges 124, anal 1–1; urosteges 89. Light yellowish brown, with twenty-six transverse deep brown rhombs across the back which terminate at the second row of scales, being separated from the back ventral cross-bar, which is opposite each, by a longitudinal yellow band. This band is not distinct between the spots. Belly strong yellow with jet black cross bars, which are on more than two gastrasteges. Tail black spotted below. A brown cross-band between the eyes. Length 27.5 inches. From Bahia, Brazil. Mus. Academy, from Dr. Otho Wucherer. This species is at first sight much like the *H. angulatus* but differs variously as above. In coloration the spots in the latter are always continued into the ventral cross-bars, and not interrupted as in *H. cyclops*.

At this writing, there are too many unknowns to make further taxonomic adjustments. As further molecular and morphological information becomes available on the type specimens (Table 1) of the species which has been placed in the synonymy of *Helicops angulatus*, the species and nomenclature will undoubtedly change again.

Facultative reproduction. The Trinidad and Venezuela populations are known to be oviparous (Mole 1924; Gorzula and Señaris 1998; Boos 2002; Ford and Ford 2002; Natera et al. 2015). One of the authors (JCM) collected a clutch of eight eggs laid in a terrestrial nest under pieces of wood and tin in November 2013. The eggs contained near full-term embryos. When combined with the February oviposition dates reported by Ford and Ford (2002), it seems likely this species reproduces year-round in Trinidad.



Fig. 6. The lectotype of *Natrrix asper* Wagler. Photos by Michael Franzen.

We also followed up on Boos' (2001) story of viviparity in a Trinidad specimen. Boos (recently deceased) attributed the story to Alan Rodriguez, an avid Trinidad snake enthusiast. One of the authors (RJA) interviewed him, but Rodriguez did not remember much about the incident related to Boos (2001), which took place about 1980. However, he reported that while searching for snakes on 15 March 2011, he observed a female *Helicops angulatus* giving birth in a drain with about 20 cm of water. The observations were made in a semi-urbanized area of Sangre Grande, Trinidad. He observed actively moving young dispersing, but several others present in the drain were stillborn. Thus, he saw this phenomenon twice (once in ~1980 and in 2011).

Curiously, Cunha and Nascimento (1981) found eggs (7–20) in 12 females from Brazil (Leste do Pará), but these authors also state that embryos were present in a single specimen. A comment in Martins and Oliveira (1998) by L.J. Vitt suggested this could be an error. Yet, according to the new evidence, it is plausible that the female examined by Cunha and Nascimento (1981) had fully developed embryos.

A related observation in the viviparous *Helicops scalaris* from the Lake Maracaibo basin (Barros et al. 2001) involved post-partum females depositing what appeared to be shell remains (Barros, pers. comm. 2020).

Table 2. A comparison of the 20 described species of *Helicops*, including some specimens placed in the synonymy of *H. angulatus* and the Trinidad populations. Scalation: AD = anterior dorsal scale rows, MD = midbody dorsal scale rows, PD = posterior body dorsal scales rows, V m/f = ventrals in males and females, sc m/f = subcaudal scales males/females; sck = subcaudal scales keeled; UL = upper labials; ULO in orbit = upper labials bordering the orbit. Reproductive modes: v = viviparity, o = oviparity, o/v both oviparity and viviparity known; ? = reproductive mode unknown. Data are based on our counts and those published in Kawashita-Ribeiro et al. (2013), Costa et al. (2016), and Moraes-Da-Silva (2019). nd = no data.

Species or population	AD	MD	PD	V = m/f	sc m/f	sck	UL	ULO	o/v
Trinidad/Paria	18–19	19	17	109–118/113–123	70–83/59–77	Yes	8	4 or 4–5	o/v?
<i>angulatus</i>	19–21	19–20	17–19	105–123/109–123	74–96/66–84	Yes	7–9	4 or 4–5	o/v
<i>cyclops</i>	nd	19	nd	124	89	Yes	8	4	?
<i>fumigatus</i>	nd	nd	nd	nd	nd	Yes	8	4	?
<i>apiaka</i>	21–24	21–22	17–19	118–127/124–132	79–103/80–84	Yes	7–9	3 or 4	?
<i>boitata</i>	25	25	21	113/nd	68/nd	No	10	34	v
<i>carinicaudus</i>	19	19	17	130–141/135–148	48–69/48–73	No	7–8	3–4, 4, 4–5	v
<i>danieli</i>	19–21	19–20	16–19	125–135/130–141	76–86/61–70	No	8–9	4	nd
<i>gomesi</i>	19	19	19	125–132/128–132	71–86/67–73	Yes	8–9	4 or 5	o
<i>hagmanni</i>	23–27	21–29	19–23	117–127/130–134	55–67/51–53	No	8	4	o
<i>infrataeniatus</i>	17–19	17–19	15–19	115–138/117–138	52–88/50–83	No	7–9	3–4, 4	v
<i>leopardinus</i>	15–22	19–22	17–19	108–126/108–130	64–89/53–76	No	8–10	3–4, 4, 3–5	v
<i>modestus</i>	19	19	17–19	112–125/116–122	54–70/53–64	No	8	3–4, 4	v
<i>nentur</i>	17	17	15	115/111–117	56/41–52	No	8	34	?
<i>pastazae</i>	23	23–25	19	121–134/130–145	93–117/72–97	No	8–10	4	?
<i>petersi</i>	21	21–23	16	135–142/137–150	85–91/67–73	No	8	4	?
<i>polylepis</i>	23–25	23–25	19	112–131/121–133	70–102/71–81	No	8–9	1–4, 1–5, 1–6	v
<i>scalaris</i>	21–25	19–21	16–19	110–119/113–125	83–95/67–81	Yes	8–9	4, 4–5	v
<i>tapajonicus</i>	19	19	17	118/121–123	79/67–76	No	8	4	?
<i>trivittatus</i>	21–25	20–23	16–19	114–128/115–129	67–80/56–66	No	8–10	4, 5	v
<i>yacu</i>	25–29	25–28	18–20	124/129–136	?/85–96	?	8–9	4, 5	?

In discussing the reproduction mode in this species, Natera et al. (2015) stated it is “vivipara lecitotrófica” (i.e., embryos receive nutrients from the yolk); and they also mentioned two females which gave birth to 21 and 22 young, in addition to a female with nine eggs in mid-development (probably referring to developing embryos). Table 2 compares the 20 known species of *Helicops* for basic meristic traits and reproductive modes.

Discussion

Helicops angulatus shows considerable intrapopulation variation in coloration and morphology (Murphy 1997; Ford and Ford 2001). Some snakes have keels on the first dorsal row of scales, and others lack them (Cope 1868). The Trinidad and Venezuela specimens we have examined all have keeled subcaudal scales. It is also clear that some *H. angulatus* have 21 dorsal scale rows at midbody, although none of the Trinidad and Venezuela specimens examined had 21 dorsal rows. Thus, Linnaeus’ *Coluber aliodras* may be the original description for a valid taxon that has 21 dorsal scale rows at mid-body. *Coluber*

surinamensis Shaw, *Natrix asper* Wagler, and *Helicops fumigatus* Cope are likely conspecific with *H. angulatus*. However, without access to the type specimens, this cannot be confirmed.

Evidence of facultative reproduction in *Helicops angulatus* was reported by Braz et al. (2016). They examined 27 gravid females, and 19 had oviductal eggs surrounded by thick, opaque, and leathery shells, indicating oviparity. The eggshell has a thick fibrous layer overlain by a thinner inorganic layer. Developing embryos were found in the eggs of five oviparous *H. angulatus* females and were visible only after eggshells were removed. They also reported six undisputable records of oviparity in *H. angulatus* that are available in the literature and another six female *H. angulatus* that were viviparous. The viviparous females had thin, transparent membranes surrounding yolk masses and embryos, and developing embryos or fully-developed young were readily visible through the extra-embryonic membranes. Embryos were partially developed in three females and near-term in a female from Colombia. Two other females contained fully-developed young. They also found two likely records of viviparity in *H. angulatus*. Two females



Fig. 7. The holotype of *Helicops fumigatus* Cope, 1868. Photo by Ned Gilmore.

had thin and transparent membranes surrounding the yolk masses, but no discernible developing embryos. Another reliable record of viviparity was obtained from a literature report of fully-developed young found in the uterus of a preserved female (Braz et al. 2016).

Braz et al. (2018) suggest eggshell thinning in *Helicops* is associated with the evolution of viviparity. They suggest eggshell reduction occurred independently in the origins of viviparity in *Helicops* and was accomplished by the loss of the mineral layer and thinning of the shell membrane. Viviparous female *H. angulatus* have a shell membrane thickness six times thinner than oviparous congeners. In contrast, the shell membrane of the viviparous *H. infrataeniatus* and *H. carinicaudus* is vestigial and 20–25 times thinner than those of their oviparous congeners. These differences suggest that eggshell reduction is a requirement for the evolution of viviparity, but a nearly complete loss of the shell membrane, as in the viviparous *Helicops*, is not.



Fig. 8. The holotype for *Helicops cyclops* Cope. Photo by Ned Gilmore.

We were skeptical of the ability of a population of snakes to contain both oviparous and viviparous females, as suggested by Alan Rodriguez's story. However, recent work on the Australian skink, *Saiphos equalis*, indicates that this ability may be widespread but undocumented in squamates. Laird et al. (2019) reported facultative oviparity by the viviparous skink, *Saiphos equalis*, which is the first report of different parity modes within a single vertebrate clutch. Eggs oviposited facultatively possess shell characteristics of both viviparous and oviparous squamates, demonstrating that the same processes produce egg coverings for both phenotypes.

Foster et al. (2019) followed up on this using transcriptomics to compare uterine gene expression in oviparous and viviparous phenotypes. They provide a molecular model for the genetic control and evolution of reproductive modes. Many genes are differentially expressed throughout the reproductive cycle of both phenotypes. Thus, viviparous and oviparous snakes

have different gene expression profiles. The differential expressions have similar biological functions which are essential for sustaining embryos, including uterine remodeling, respiratory gas and water exchange, and immune regulation. As might be expected, the similarities suggest long egg-retention is an exaptation for viviparity; or it reflects the parallel evolution of similar changes in gene expression needed for long egg-retention oviparous species. In contrast, changes in gene expression across the reproductive cycle of the long egg retaining oviparous *Saiphos equalis* are dramatically different from those of oviparous skinks. This supports the assertion that the oviparous *S. equalis* exhibit a phenotype that is intermediate between true oviparity and viviparity.

The ability of *Saiphos equalis* to change reproductive modes suggests to us that the Trinidad *Helicops angulatus* population (and likely other mainland *H. angulatus* populations) also has this capability. Trinidad *Helicops* likely have two reproductive phenotypes, making this population incredibly valuable to science, for unraveling a better understanding of the evolution of viviparity in Squamata.

Conservation. Given the highly aquatic habits of these snakes, habitat destruction and water pollution are likely the main threats to their survival. They are found in coastal areas as well as inland waters. Coastal mangrove forests are changing in complex ways, with deforestation combined with new growth (Al-Tahir and Baban 2005; Juman and Ramsewak 2013). Trinidad coastal areas have significant oil pollution from the thousands of large vessels that move through Trinidad waters annually (Water Resource Agency 2001).

Freshwater pollutants originate from urban, domestic, and industrial waste, agricultural chemicals, as well as sediments and oil spills. Lowering the water table to a level which exceeds the aquifer's replenishment abilities has resulted in brackish water intrusion into the El Socorro aquifers (Water Resource Agency 2001). Nitrate and bacterial contamination result from the excessive use of agrochemicals, leaking septic tanks, wastes from livestock, and agro-industrial effluents such as pesticides and fertilizers. Specifically, Trinidad has a severe problem with the excessive use of certain fertilizers and pesticides and the release of high concentrations of waste from intensive animal farm operations. Sewage and solid wastes are severe in some areas, such as the Beetham/Laventille swamp (north of the Caroni River). Tires, motor vehicles, major appliances, floating livestock, and an array of consumer disposables are often deposited in the swamp (Water Resource Agency 2001).

Deforestation in the Northern Range removes the protective vegetation layer, resulting in an excessive run-off that exacerbates flooding in the rainy season. Of great concern is the increase in residential development in watershed areas that significantly impacts the run-off rates, sedimentation levels of rivers, and downstream

flooding. Silt from quarries has raised the substrates at the lower reaches and mouth of the Caroni River, affecting the hydrology of the river. High concentration rates of siltation affect rivers, such as the North Oropuche and Aripo Rivers in the northeast.

Saaristo et al. (2018) demonstrated how chemical contaminants (e.g., metals, pesticides, and pharmaceuticals) are changing ecosystems by altering animal behavior through physiological changes. Their framework shows how the sublethal behavioral effects of pollutants can have a mixture of negative, and sometimes positive, changes that vary dynamically within the same individuals and populations.

Of less concern are the snakes taken as by-catch by fishers. Hernández-Ruiz et al. (2014) used hoop nets to sample turtle populations in northern Brazil and obtained a by-catch of *Helicops angulatus*. However, through discussions with fishers in Nariva Swamp, we (JCM, RJA) found that they usually release the snakes captured in fishing nets. The loss of the unique *Helicops angulatus* populations on Trinidad and elsewhere would be a wasted significant opportunity to expand our understanding of the evolution of reproductive modes in the Squamata.

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Literature Cited

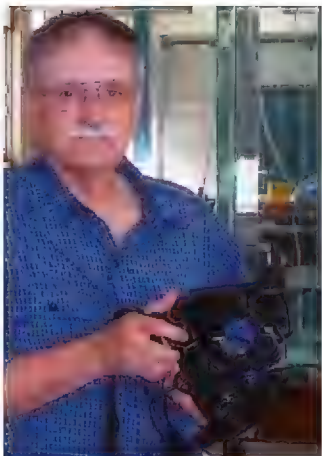
- Al-Tahir R, Baban SM. 2005. An evaluation of recent changes in mangrove forest habitats in Trinidad West Indies. *Journal of Tropical Biodiversity* 8(3): 187–198.
- Andersson LG. 1899. Catalogue of the Linnaean type-specimens of snakes in The Royal Museum in Stockholm. *Bihang till Kongliga Svenska Vetenskaps-Akademiens Handlingar* 24(4:6): 1–35.
- Antonelli A, Quijada-Mascareñas A, Crawford AJ, Bates JM, Velasco PM, Wüster W. 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. Pp. 386–404 In: *Amazonia, Landscape and Species Evolution: a Look into the Past*. Editors, Hoorn C, Wesselingh F. Wiley-Blackwell, Chichester, United Kingdom. 447 p.
- Barros T, López JC, Alvarado M. 2001. *Helicops scalaris*. Reproduction. *Herpetological Review* 32: 47.
- Bauer AM, Wahlgren R. 2013. On the Linck collection and specimens of snakes figured by Johann Jakob

- Scheuchzer (1735), the oldest fluid-preserved herpetological collection in the world. *Bonn Zoological Bulletin* 62: 220–252.
- Blackburn DG. 1994. Standardized criteria for the recognition of embryonic nutritional patterns in squamate reptiles. *Copeia* 1994: 925–935.
- Blackburn DG. 2000. Reptilian viviparity: past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology A* 127(4): 391–409.
- Blackburn DG. 2006. Squamate reptiles as model organisms for the evolution of viviparity. *Herpetological Monographs* 20: 131–146.
- Boos HEA. 2001. *The Snakes of Trinidad and Tobago*. Texas A&M Press, College Station, Texas, USA. 270 p.
- Braz HB, Almeida-Santos SM, Murphy CR, Thompson MB. 2018. Uterine and eggshell modifications associated with the evolution of viviparity in South American water snakes (*Helicops* spp.). *Journal of Experimental Zoology B* 330(3): 165–180.
- Braz HB, Scartozzoni RR, Almeida-Santos SM. 2016. Reproductive modes of the South American water snakes: a study system for the evolution of viviparity in squamate reptiles. *Zoologischer Anzeiger* 263: 33–44.
- Burbrink FT, Lawson R, Slowinski BB. 2000. Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* 54: 2,107–2,118.
- Cisneros-Heredia DF. 2006. Reptilia, Colubridae, *Helicops angulatus* and *Helicops leopardinus*: distribution extension, new country record. *Check List* 2(1): 36–37.
- Cole CJ, Townsend CR, Reynolds RP, MacCulloch RD, Lathrop A. 2013. Amphibians and reptiles of Guyana, South America: illustrated keys, annotated species accounts, and a biogeographic synopsis. *Proceedings of the Biological Society of Washington* 125: 317–620.
- Colonnello G. 2004. Las planicies deltaicas del río Orinoco y Golfo de Paria: aspectos físicos y vegetación. Pp. 37–54 In: *Evaluación Rápida de la Biodiversidad y Aspectos Sociales de los Ecosistemas Acuáticos del Delta del Río Orinoco y Golfo de Paria, Venezuela*. Boletín RAP de Evaluación Biológica 37. Editors, Lasso CA, Alonso LE, Flores AL, Love G. Conservation International, Washington, DC, USA. 358 p.
- Cope ED. 1868. Sixth contribution to the herpetology of tropical America. *Proceedings of the Academy of Natural Science of Philadelphia* 20: 305–313.
- Costa HC, Santana DJ, Leal F, Koroiva R, Garcia PC. 2016. A new species of *Helicops* (Serpentes: Dipsadidae: Hydropsini) from southeastern Brazil. *Herpetologica* 72(2): 157–166.
- Cunha OR, Nascimento FP. 1978. Ofídios da Amazônia X. As cobras da região leste do Pará. *Publicações Avulsas do Museo Goeldi* 31: 1–218.
- Cunha OR, Nascimento FP. 1981. Ofídios da Amazônia XII. Observações sobre a viviparidade em ofídios do Pará e Maranhão (Ophidia: Aniliidae, Boidae, Colubrida e Viperidae). *Boletim do Museo Paraense Emílio Goeldi* 109: 1–20.
- Cunha OR, Nascimento FP. 1993. Ofídios da Amazônia. As cobras da região leste do Pará. *Boletim do Museo Paraense Emílio Goeldi (Série Zoologia)* 9: 1–191.
- Daza JM, Smith EN, Pez VP, Parkinson CL. 2009. Complex evolution in the Neotropics: the origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae). *Molecular Phylogenetics and Evolution* 53: 653–667.
- de Carvalho Teixeira C, de Assis Montag LF, dos Santos-Costa MC. 2017. Diet composition and foraging habitat use by three species of water snakes, *Helicops* Wagler, 1830 (Serpentes: Dipsadidae), in eastern Brazilian Amazonia. *Journal of Herpetology* 51(2): 215–222.
- Di Pietro D, Alcalde L, Williams J. 2014. New cranial characters in the tribe Hydropsini (Serpentes: Dipsadidae: Xenodontinae). *Acta Herpetologica* 9(1): 1–4.
- Di Pietro DO, Alcalde L, Williams JD. 1974. Nasal cartilages, hyobranchial apparatus, larynx, and glottal tubes in four species of Hydropsini (Serpentes: Dipsadidae: Xenodontinae). *Veterbrate Zoology* 64: 103–111.
- Dowling HG. 1975. The Nearctic snake fauna. Pp. 191–202 In: *1974 Yearbook of Herpetology*. Editor, Dowling HG. Herpetological Information Search System Publications, New York, New York, USA. 256 p.
- Ford NB, Ford DF. 2002. Notes on the ecology of the South American water snake, *Helicops angulatus* (Squamata: Colubridae), in Nariva Swamp, Trinidad. *Caribbean Journal of Science* 38(1/2): 129–131.
- Foster CS, Thompson MB, Van Dyke JU, Brandley MC, Whittington CM. 2020. Emergence of an evolutionary innovation: gene expression differences associated with the transition between oviparity and viviparity. *Molecular Ecology* 29(7): 1,315–1,327.
- França FG, Venâncio NM. 2010. Reptiles and amphibians of a poorly known region in southwest Amazonia. *Biotemas* 23(3): 71–84.
- França RC, Germano CE, França FG. 2012. Composition of a snake assemblage inhabiting an urbanized area in the Atlantic Forest of Paraíba State, northeast Brazil. *Biota Neotropica*. 2012(3): 183–195.
- Froese R, Garilao CV. 2019. *Cichlasoma bimaculatum* (Linnaeus, 1758), Black acara. FishBase. Available: <http://www.fishbase.org> [Accessed: 25 February 2020].
- Gorzula S, Señaris JC. 1998. Contribution to the herpetofauna of the Venezuelan Guyana I. A database. *Scientia Guianae* 8: 1–269.
- Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4.

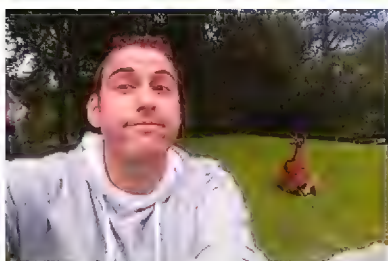
- A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27: 221–224.
- Gray JE. 1849. *Specimens of Snakes in the Collection of the British Museum*. Edward Newman, London, United Kingdom. 125 p.
- Grazziotin FG, Zaher H, Murphy RW, Scrocchi G, Benavides MA, Zhang YP, Bonatto SL. 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28(5): 437–459.
- Guimarães JA, Dias EJ, Oliveira AR. 2010. *Helicops angulatus* (Watersnake). Diet and reproduction. *Herpetological Review* 41(1): 93.
- Hernández-Ruiz EJ, Wariss Figueiredo MA, Brito Pezzuti JC. 2014. Bycatch of *Helicops angulatus* (Linnaeus 1758) (Reptilia: Squamata: Colubridae) in hoop-traps used to capture fresh water turtles on the coast of Pará, Brazil. *Acta Biológica Colombiana* 19(1): 119–120.
- Hodges WL. 2004. Evolution of viviparity in horned lizards (*Phrynosoma*): testing the cold-climate hypothesis. *Journal of Evolutionary Biology* 17(6): 1,230–1,237.
- Hoogmoed MS, Gruber U. 1983. Spix and Wagler type specimens of reptiles and amphibians in the Natural History Museum in Munich (Germany) and Leiden (The Netherlands). *Spixiana Supplement* 9: 319–415.
- Husson AM. 1978. *The Mammals of Suriname*. Brill, Leiden, Netherlands. 729 p.
- Juman R, Ramsewak D. 2013. Status of mangrove forests in Trinidad and Tobago, West Indies. *Caribbean Journal of Science* 47(2–3): 291–304.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3,059–3,066.
- Kawashita-Ribeiro RA, Ávila RW, Morais DH. 2013. A new snake of the genus *Helicops* Wagler, 1830 (Dipsadidae, Xenodontinae) from Brazil. *Herpetologica* 69(1): 80–90.
- Kelly CM, Barker NP, Villet MH. 2003. Phylogenetics of advanced snakes (Caenophidia) based on four mitochondrial genes. *Systematic Biology* 52(4): 439–459.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86(16): 6,196–6,200.
- Laird MK, Thompson MB, Whittington CM. 2019. Facultative oviparity in a viviparous skink (*Saiphos equalis*). *Biology Letters* 15(4): 20180827.
- Lambert SM, Wiens JJ. 2013. Evolution of viviparity: a phylogenetic test of the cold-climate hypothesis in phrynosomatid lizards. *Evolution* 67(9): 2,614–2,630.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.
- Larkin MA, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21): 2,947–2,948.
- Lasso CA, Sánchez-Duarte P. 2011. *Los Peces del Delta del Orinoco. Diversidad, Bioecología, Uso y Conservación*. Fundación La Salle de Ciencias Naturales y Chevron CA Venezuela, Caracas, Venezuela. 500 p.
- Lasso CA, Rial A, Colonnello G, Machado-Allison A, Trujillo F. (Editors). 2014. *XI. Humedales de la Orinoquia (Colombia-Venezuela). Serie Editorial Recursos Hidrobiológicos y Pesqueros Continentales de Colombia*. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH), Bogotá, Colombia. 303 p.
- Latrubesse EM, Arima EY, Dunne T, Park E, Baker VR, d'Horta FM, Wight C, Wittmann F, Zuanon J, Baker PA, et al. 2017. Damming the rivers of the Amazon basin. *Nature* 546(7658): 363–369.
- Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 37(2): 581–601.
- Linnaeus C. 1758. *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata*. Laurentii Salvii, Stockholm, Sweden. 824 p.
- Ma L, Buckley LB, Huey RB, Du WG. 2018. A global test of the cold-climate hypothesis for the evolution of viviparity of squamate reptiles. *Global Ecology and Biogeography* 27(6): 679–689.
- Martins M, Oliveira ME. 1998. Natural history of snakes in forests of the Manaus region, Central Amazonia, Brazil. *Herpetological Natural History* 6: 78–150.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *Gateway Computing Environments Workshop, 14 November 2010, New Orleans, Louisiana*. IEEE, Piscataway, New Jersey, USA.
- Mohammed RS, Manickchan SA, Charles SP, Murphy JC. 2014. The herpetofauna of southeast Trinidad, Trinidad and Tobago. *Living World* 2014: 12–20.
- Mole RR. 1924. The Trinidad snakes. *Proceedings of the Zoological Society of London* 94(1): 235–278.
- Moraes-Da-Silva A, Amaro RC, Nunes PM, Strüssmann CH, Teixeira MJ, Andrade AJ, Curcio FF. 2019. Chance, luck, and a fortunate finding: a new species of watersnake of the genus *Helicops* Wagler, 1828 (Serpentes: Xenodontinae), from the Brazilian

- Pantanal wetlands. *Zootaxa* 4651(3): 445–470.
- Murphy JC. 2012. Marine invasions by non-sea snakes, with thoughts on terrestrial-aquatic-marine transitions. *Integrative and Comparative Biology* 52: 217–226.
- Natera Mumaw M, Esqueda González LF, Castelaín Fernández M. 2015. *Atlas Serpientes de Venezuela. Una Visión Actual de su Diversidad*. Dimacofi Negocios Avanzados S.A., Santiago, Chile. 441 p.
- Neill WT. 1964. Viviparity in snakes: some ecological and zoogeographical considerations. *The American Naturalist* 98(898): 35–55.
- Nogueira CC, Argôlo AJ, Arzamendia V, Azevedo JA, Barbo FE, Bérnils RS, Bolochio BE, Borges-Martins M, Brasil-Godinho M, Braz H, et al. 2019. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. *South American Journal of Herpetology* 14(Suppl 1): 1–274.
- Novoa D. 2000. *La Pesca en el Golfo de Paria y Delta del Orinoco Costero*. CONOCO Venezuela, Editorial Arte, Caracas, Venezuela. 140 p.
- Oberdorff T, Dias MS, Jézéquel C, Albert JS, Arantes CC, Bigorne R, Carvajal-Valleros FM, De Wever A, Frederico RG, Hidalgo M, et al. 2019. Unexpected fish diversity gradients in the Amazon basin. *Science Advances* 5(9): p.eaav8681.
- Packard GC, Tracy CR, Roth JJ. 1977. Physiological ecology of reptilian eggs and embryos, and evolution of viviparity within class Reptilia. *Biological Reviews of the Cambridge Philosophical Society* 52: 71–105.
- Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice J, Grabowski G. 1991. *The Simple Fool's Guide to PCR*. University of Hawaii Press, Honolulu, Hawaii, USA. 45 p.
- Pérez-Santos C, Moreno AG. 1988. Ofidios de Colombia. *Monografie Museo Regionale di Scienze Naturali-Torino* 6: 1–517.
- Qualls CP, Andrews RM. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor quality offspring in the lizard, *Sceloporus virgatus*. *Biological Journal of the Linnean Society* 67: 353–376.
- Ribeiro SC, Roberto IJ, Sales DL, Ávila RW, Almeida WD. 2012. Amphibians and reptiles from the Araripe bioregion, northeastern Brazil. *Salamandra* 48(3): 133–146.
- Roberto IJ, Ribeiro SC, de Sousa Delfino MM, de Oliveira Almeida W. 2009. Reptilia, Colubridae, *Helicops angulatus*: distribution extension and rediscovery in the state of Ceará. *Check List* 5(1): 118–121.
- Rocha R, López-Baucells A. 2014. Predation attempt of *Hypsiboas boans* (Anura: Hylidae) by *Helicops angulatus* (Squamata: Dipsadidae) with notes on defensive behavior. *Alytes* 30(1–4): 78–81.
- Rodriguez-Diaz T, Brana F. 2012. Altitudinal variation in egg retention and rates of embryonic development in oviparous *Zootoca vivipara* fit predictions from the cold-climate model on the evolution of viviparity. *Journal of Evolutionary Biology* 25(9): 1,877–1,887.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1,572–1,574.
- Rossman DA. 1973. Miscellaneous notes on the South American water snake genus *Helicops*. *HISS News-Journal* 1(6): 189–191.
- Rossman DA. 1984. *Helicops angulatus* (South American water snake). Reproduction. *Herpetological Review* 15: 50.
- Rossman DA. 2002. Morphological variation in the endemic Colombian water snake, *Helicops danieli* Amaral, 1937 (Serpente: Xenodontidae). *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 101: 589–594.
- Roze JA. 1957a. Notas sobre *Hydrops lehmanni* Dunn, 1944, y los géneros neotropicales: *Pseudoeryx*, *Hydrops*, y *Helicops* (Colubridae). *Acta Biológica Venezolánica* 2: 17–26.
- Roze JA. 1957b. Resumen de una revisión del género *Hydrops* Wagler, 1830 (Serpentes: Colubridae). *Acta Biológica Venezolánica* 2: 51–95.
- Saaristo M, Brodin T, Balshine S, Bertram MG, Brooks BW, Ehlman SM, McCallum ES, Sih A, Sundin J, Wong BB, et al. 2018. Direct and indirect effects of chemical contaminants on the behavior, ecology, and evolution of wildlife. *Proceedings of the Royal Society B: Biological Sciences* 285(1885): 20181297.
- Schlegel H. 1837. *Essai sur la Physionomie des Serpens*. M.H. Schonekat, Amsterdam, Netherlands. 251 p.
- Sergeev A. 1940. Researches in the viviparity of reptiles. *Moscow Society of Naturalists* (Jubilee Issue): 1–34.
- Shaw G. 1802. *General Zoology, or Systematic Natural History*. Volume 3, part 2. G. Kearsley, Thomas Davison, London, United Kingdom. 313–615 p.
- Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. Pp. 605–694 In: *Biology of the Reptilia, Volume 15*. Editors, Gans C, Billet F. Wiley, New York, New York, USA. 731 p.
- Shine R, Bull J. 1979. The evolution of live-bearing in lizards and snakes. *The American Naturalist* 113: 905–923.
- Sioli H. 1975. Tropical rivers as expressions of their terrestrial environments. Pp. 275–288 In: *Tropical Ecological Systems. Trends in Terrestrial and Aquatic Research*. Editors, Goley F, Medina E. Springer-Verlag, New York, New York, USA. 398 p.
- Silvestro D, Michalak I. 2012. RaxmlGUI: a graphical front-end for RAxML. *Organisms, Diversity, and Evolution* 12: 335–337.
- Starace F. 2013. *Serpents et Amphisbènes de Guyane Française*. Ibis Rouge Éditions, Matoury, French Guiana. 604 p.
- Tinkle DW, Gibbons JW. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications Museum of Zoology University of*

- Michigan 154: 1–55.
- Tisseuil C, Cornu JF, Beauchard O, Brosse S, Darwall W, Holland R, Hugueny B, Tedesco PA, Oberdorff T. 2013. Global diversity patterns and cross-taxa convergence in freshwater systems. *Journal of Animal Ecology* 82: 365–376.
- Uetz P, Freed P, Hošek J. (Editors). 2020. The Reptile Database, *Helicops angulatus*. Available: <http://www.reptile-database.org> [Accessed: 25 February 2020].
- Vidal N, Dewynter M, Gower DJ. 2010. Dissecting the major American snake radiation: a molecular phylogeny of the Dipsadidae Bonaparte (Serpentes, Caenophidia). *Comptes Rendus Biologies* 333(1): 48–55.
- Vidal N, Kindl SG, Wong A, Hedges SB. 2000. Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution* 14(3): 389–402.
- Wagler JG. 1824. *Serpentum Brasiliensium species nova ou histoire naturelle des espèce nouvelle des serpens recueillies et observées pendant le voyage dans l'interieur du Brésil dans le années 1817, 1818, 1819, 1820 exécuté par ordre de Sa Majesté le Roi de Bavière, publiée par Jean Spix. Monachii. Typis F.S. Hübschmanni, Munich, Germany. VIII, 75 p.*
- Wagler J. 1828–1833. *Descriptiones et Icones Amphibiorum. Tres Partes Cum XXXVI Tabulis*. J.G. Cotta, Munich, Stuttgart, and Tübingen, Germany.
- Wagler JG. 1830. *Natürliches System der Amphibien, mit vorangehender Classification der Säugetiere und Vögel. Ein Beitrag zur vergleichenden Zoologie. 1.0*. J.G. Cotta, Munich, Stuttgart, and Tübingen, Germany. 354 p.
- Wallach V, Williams KL, Boundy J. 2014. *Snakes of the World: a Catalogue of Living and Extinct Species*. CRC Press/Taylor and Francis, New York, New York, USA. 1,237 p.
- Water Resource Agency. 2001. *National Report in Integrating the Management of Watersheds and Coastal Areas in Trinidad and Tobago*. Prepared for The Ministry of the Environment, Port of Spain, Trinidad and Tobago. 125 p.
- Weekes HC. 1935. A review of placentation among reptiles, with particular regard to the function and evolution of the placenta. *Proceedings of the Zoological Society of London* 105(3): 625–645.
- Zaher H, Grazziotin FG, Cadle JE, Murphy RW, Moura-Leite JC, Bonatto SL. 2009. Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American xenodontines: a revised classification and descriptions of new taxa. *Papéis Avulsos de Zoologia* 49(11): 115–153.
- Zaher H, Yáñez-Muñoz MH, Rodrigues MT, Graboski R, Machado FA, Altamirano-Benavides M, Bonatto SL, Grazziotin FG. 2018. Origin and hidden diversity within the poorly known Galápagos snake radiation (Serpentes: Dipsadidae). *Systematics and Biodiversity* 16(7): 614–642.



John C. Murphy is a naturalist who focuses on snakes. When he is not hiking in the desert or examining specimens in the lab, John is often writing about reptiles. He is a retired science educator who got serious about his lifelong fascination with lizards and snakes in the early 1980s, when he and his family made their first trip to Trinidad. The work on Trinidad and Tobago provided valuable lessons that shaped his views of nature and evolution. Today he is still working on the eastern Caribbean herpetofauna. In the 1990s he worked on homalopsid snakes in Southeast Asia with other researchers from the Field Museum (Chicago, Illinois, USA). Today John resides in southeastern Arizona (USA) and is involved in multiple projects that concern arid habitats and the impact of climate change on biodiversity. His most recent book, with co-author Tom Crutchfield, is *Giant Snakes, A Natural History*. Born and raised in Joliet, Illinois, he first learned about reptiles on his grandfather's farm by watching Eastern Garter Snakes emerge from their winter dens and Snapping Turtles depositing their eggs at the edge of a cattail marsh.



Antonio Muñoz-Mérida is a bioinformatician with a background in biology and genetics. During his Ph.D. work, Antonio developed several bioinformatics tools and gained computing skills that have been improved during his post-doctoral appointment as the main bioinformatician at the Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO) research center in Portugal. His expertise ranges across most of the OMICs associated with Next Generation Sequencing and functional annotation of whole genomes.



Renoir J. Auguste is a Trinidad and Tobago herpetologist. Renoir received his M.Sc. in Biodiversity Conservation from The University of the West Indies, St. Augustine Campus, Trinidad and Tobago, and is interested in the ecology and conservation of amphibians and reptiles. He has conducted herpetological surveys across Trinidad and Tobago professionally for national baseline surveys aimed at improving protected areas, as part of his academic degrees. He has also conducted surveys as part of his academic degree work and voluntarily with the local environmental NGO Trinidad and Tobago Field Naturalists' Club, in which he held the position as president for three years.



Oscar Miguel Lasso-Alcalá is Curator of the Fish Collection and a Senior Researcher at Museo de Historia Natural La Salle (MHNLS), Fundación La Salle de Ciencias Naturales, Caracas, Venezuela. His undergraduate studies were in Fishing Technology, Aquaculture, and Oceanography, followed by postgraduate work in Agricultural Zoology and Estuary Ecology. He is primarily an ichthyologist, with more than 30 years of experience in the taxonomy, biology, ecology, and fisheries aspects of marine, estuarine, and freshwater fauna. However, during more than 60 research projects and 230 scientific expeditions, he has participated in the study of biodiversity, including amphibians and reptiles. In this way, he has collaborated in several studies of the herpetofauna in the Caribbean, and different regions of Venezuela, where a frog (*Tachiramantis lassoalcalai*) was described in his honor.



Gilson A. Rivas was born in Caracas, Venezuela. He currently serves as co-editor of the scientific journal *Anartia*, and is a collection manager at the Museo de Biología de la Universidad del Zulia, Maracaibo, a Venezuelan centennial university that began academic activities on 11 September 1891. For over two decades, Gilson has devoted his studies to the taxonomy and conservation of the neotropical herpetofauna, and has authored or co-authored more than 100 academic publications, describing over 30 new species of amphibians and reptiles, and a new genus of dipsadine snakes, *Plesiodipsas*. Gilson is the author (with G. Ugueto) of the book *Amphibians and Reptiles of Margarita, Coche, and Cubagua*; and together with M. De Freitas, H. Kaiser, C.L. Barrio-Amorós, and T.R. Barros produced *Amphibians of the Península de Paria: a Pocket Field Guide*. Gilson’s research interests are focused on the herpetofauna of the Venezuelan coastal range and insular ecosystems, as well as the influences of invasive species and human development and their impact on the native fauna.



Michael J. Jowers is an evolutionary biologist with broad interests in the processes and timing of speciation. His work focuses on tropical island biogeography, phylogeography, systematics, population genetics, taxonomy, and conservation. Michael is deeply involved in amphibian and reptile studies from the islands of Trinidad and Tobago (Lesser Antilles), but he is also interested in other organisms such as birds, mammals, and insects; and he actively leads studies throughout South America, Africa, Europe, and Asia.

Supplementary Material

Table S1. Primers used in gene fragment amplification.

Gene	Primer name and sequence	Reference
12S rDNA	12SA 5'- AAAGTGGGATTAGATACCCCACTAT -3'	Kocher et al. 1989
12S rDNA	12SB 5'- GAGGG TGACGGGCGGTGTGT -3'	Kocher et al. 1989
16S rDNA	16SL 5'- GCCTGTTTATCAAAAACAT -3'	Palumbi et al. 1991
16S rDNA	16SH 5'- CCGGTCTGAACTCAGATCACGT - 3'	Palumbi et al. 1991
cytb	14910 5'- GACCTGTGATMTGAAAAACCAATCG -3'	Burbrink et al. 2000
cytb	H16064 5'- CTTTGGTTTACAAGAACAATGCTT -3'	Burbrink et al. 2000
c-mos	S77 5'- CATGGACTGGGATCAGTTATG - 3'	Lawson et al. 2005
c-mos	S78 5'- CCTTGGGTGTGATTTTCTCACCT - 3'	Lawson et al. 2005

Table S2. GenBank accession numbers of species and specimens of *Helicops* and outgroup taxa included in the molecular phylogenetic reconstructions and genetic divergence analyses. *Helicops angulatus* from this study are all from Trinidad (West Indies): UWIZM.2015.18.32 (Rd. Kernahan to Bush Bush), UWIZM.2011.20.22 (Nariva Swamp), UWIZM.2013.6 (Nariva Swamp), CAS231757 (Nariva Road, Manzanilla Beach), CAS231758 (Nariva Road, Manzanilla Beach), and CAS231760 (Nariva Road, Manzanilla Beach).

Species	12S rDNA	16S rDNA	c-mos	Cyt b
<i>Pseudoeryx plicatilis</i> (PSPL001)	GQ457826	GQ457765	GQ457886	-
<i>Pseudoeryx plicatilis</i> (H1399)	MN038102	MN038115	MN032460	-
<i>Hydrops triangularis</i> (H1476)	MN038103	MN038114	MN032461	-
<i>Hydrops triangularis</i> (HYTR001)	GQ457804	GQ457744	GQ457864	-
<i>Helicops leopardinus</i> (UFMTR11939)	MN038108	MN038121	MN032465	-
<i>Helicops leopardinus</i> (H1733)	-	MN038120	MN032464	-
<i>Helicops modestus</i> (MTR11762)	MN038109	MN038122	MN032468	-
<i>Helicops modestus</i> (MTR19715)	MN038110	MN038123	MN032469	-
<i>Helicops pictiventris</i> (HEPI001)	GQ457800	GQ457741	GQ457860	-
<i>Helicops infrataeniatus</i> (HIN001)	GQ457799	GQ457740	GQ457859	-
<i>Helicops boitata</i> (UFMTR11940)	MN038112	MN038124	MN032471	-
<i>Helicops carinicaudus</i> (142)	MN038104	MN038125	MN032462	-
<i>Helicops nentur</i> (DJS-2016)	-	KT453992	KT453991	-
<i>Helicops polylepis</i> (H919)	MN038111	-	MN032470	-
<i>Helicops hagmanni</i> (MTR12961)	MN038106	MN038118	MN032467	-
<i>Helicops hagmanni</i> (MTR13320)	MN038107	MN038119	MN032466	-
<i>Helicops gomesi</i> (HEGO001)	GQ457798	GQ457739	GQ457858	-
<i>Helicops gomesi</i> (141)	MN038105	MN038117	MN032463	-
<i>Helicops angulatus</i> (HEAN001)	GQ457797	GQ457738	GQ457857	-
<i>Helicops angulatus</i> (MRT7588)	MN038113	MN038116	MN032472	-
<i>Helicops angulatus</i> (CAS231758)	MT951589	MT951597	MT951603	MT951607
<i>Helicops angulatus</i> (CAS231757)	MT951591	MT951599	MT951605	MT951608
<i>Helicops angulatus</i> (CAS231760)	MT951590	MT951600	MT951604	MT951606
<i>Helicops angulatus</i> (UWIZM.2013.6)	MT951592	MT951595	MT951602	-
<i>Helicops angulatus</i> (UWIZM.2015.18.32)	MT951593	MT951598	-	MT951609
<i>Helicops angulatus</i> (UWIZM.2011.20.22)	MT951594	MT951596	MT951601	MT951610



Cannibalism in the High Andean Titicaca Water Frog, *Telmatobius culeus* Garman, 1875

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Abstract.—Cannibalism has been considered as an aberrant behavior, but in amphibians and reptiles, it could play a role in the biology of a population. This paper reports conspecific predation in the Titicaca Water Frog (*Telmatobius culeus*), as the first record of cannibalism of adults in this genus. Heterocannibalism describes cases where adults eat larvae, juveniles, and adults. The phenotypical differences between predator and prey suggest this is a case of cannibalistic polyphenism, where cannibalistic morphs seem to have features that facilitate the predation of the conspecifics. Both females and males were observed to be cannibalistic, and suggestions are proposed regarding why both sexes could benefit from cannibalism, as well as how a high density of a fully aquatic species that shares the habitat, resources, and refuges with other conspecifics increases the chances of encounters and cannibalism.

Keywords. Amphibia, conspecific predation, Critically Endangered, heterocannibalism, anurophagy, size relationships

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Introduction

Intraspecific predation, or cannibalism, is the process of eating an individual of the same species and it is widespread in the animal kingdom (Fox 1975; Polis 1981). It may be important in the population ecology of certain amphibians, where in some genera, such as *Rana* and *Notophthalmus*, conspecifics are reported to consist of 7–25% of all diet items (Polis and Myers 1985). Some of the factors that may stimulate cannibalism include intraspecific predation, environmental and nutritional stress, and high densities, and it may also be part of a reproductive strategy (Fox 1975; Kaplan and Sherman 1980; Polis 1981; Polis and Myers 1985).

In amphibians, as in reptiles, the groups that are more often cannibalized tend to be younger and smaller animals (Polis and Myers 1985). However, in contrast to reptiles, cannibalism among amphibians appears to be important in the biology of these species. Cannibalism may be an important strategy for larvae living in ephemeral habitats, where the pressure on this stage is very high, and the first individuals that will metamorphose and emerge from water are mainly the cannibal morph (Crump 1983). Juveniles are commonly eaten by adult frogs, and this predation of juveniles could be a strategy to remove future competitors for the

predator itself and for its own offspring (Kaplan and Sherman 1980). Juveniles are more often cannibalized before they reach a certain size. Toledo et al. (2007) explain the term of “status inversion,” i.e. the process of turning from prey into predator as anurans increase in body size. Examples of status inversion have been noted in *Conraua*, *Ceratophrys*, some *Leptodactylus*, *Pyxicephalus*, and *Lithobates*, with adults that can prey upon several types of small vertebrates and even conspecifics (Duellman and Trueb 1994). Cannibalism between adult individuals is less frequent and, as explained by Measey et al. (2015), the prey size of a conspecific adult could deliver some negative effects and even the death of the predator. Few reports of conspecific predation of adults are known, with just a couple of examples such as *Ceratophrys* and *Lepidobatrachus llanensis* (Cochran 1955; Hulse 1978; Polis 1981).

Polyphenism is the occurrence of alternative phenotypes in a population that are produced from a single genotype in response to different environmental stimuli (West-Eberhard 1989). The phenomenon of cannibalistic polyphenism and its causes were reviewed by Crump (1983, 1992) and Hoffman and Pfennig (1999), with reference to phenotypic differences in behavior, morphology, growth, or life history between

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cannibal and non-cannibal forms and often resulting in expression of the most advantageous phenotype under current environmental conditions (Hoffman and Pfennig 1999). These adaptations include rapid development, larger size, hypertrophied jaw musculature, and more aggressive behavior, among other factors that could facilitate the cannibalism in these morphs.

Lake Titicaca Frog is considered an iconic species, and as one of the largest fully aquatic frogs in the world (Fontúrbel 2009; De La Riva 2005) it has several adaptations to survive in the conditions that a high Andean lake provides. For many years, the taxonomy of the four species of *Telmatobius* described in the Titicaca basin (*Telmatobius albiventris*, *Telmatobius crawfordi*, *Telmatobius culeus*, and *Telmatobius marmoratus*) has been chaotic. In this area, as many as four subspecies of *T. albiventris*, six of *T. culeus*, two of *T. crawfordi*, and four of *T. marmoratus* have been described, bringing the total number of taxa to 16 subspecies belonging to four putative species (De La Riva 2005). As part of the taxonomic revision, Benavides et al. (2002) demonstrated that *T. albiventris*, *T. culeus*, and *T. crawfordi* represent a single taxon, and that *T. culeus* varies noticeably in morphology and body size from *T. marmoratus*. Benavides (2005) suggested the absence of reciprocal monophyly for the two species present in the lake, recognized as *T. culeus sensu lato* and *T. marmoratus*. He also indicated that lacustrine haplotypes are much older than riverine ones, in agreement with the findings of De la Riva et al. (2010) and also as indicated by Aguilar and Valencia (2009). Consequently, *T. culeus* is the only species present in lacustrine habitats.

This report provides the first evidence of cannibalism in Titicaca Water Frog (*Telmatobius culeus*), a Critically Endangered anuran of the High Andes which is fully aquatic and endemic to Lake Titicaca and its surroundings (De La Riva 2005).

Materials and Methods

During November 2008 to December 2015 studies to monitor the species were conducted at different localities on the Bolivian side of Lake Titicaca, Department of La Paz. Monitoring consisted of swimming on the surface of the water with a snorkel, and counting and observing individuals at depths between 0.5 and 7 m. In some cases when an individual was observed, immersions with the snorkel up to 6 m were carried out to capture the frogs. In addition, scuba diving observations were carried out at depths of up to 12 m for longer periods. When individuals were captured, Snout-Vent Length (SVL) and body mass were obtained together with other biotic and abiotic information. Because this species is Critically Endangered, live frogs were not collected. Casually observed individuals were kept for limited time for specific measurements and then returned to the lake.

Results and Discussion

During the study period, three records of wild individuals eating conspecifics were observed, in addition to similar observations of three others in captivity.

- On 20 January 2009 at 1147 h, in the locality of Patapatani, Bolivia (16°4'58.58"S, 69°7'45.47"W) a male individual (Fig. 1a) of *Telmatobius culeus* was captured at a depth of 5.6 m. This individual was maintained alone during one night in an aquarium, and the next day at 0730 h the frog excreted a juvenile of *T. culeus* with the entire body digested except for the bones and some soft tissue.
- The second case occurred in Sicuani, Bolivia (16°5'23.09"S, 69°6'48.50"W) on 4 November 2011. A female individual was captured at a depth of 4 m. After some measurements, it was separated in a container with water for about half an hour. Within that period a sub-adult male individual was vomited up with the head partially digested.
- On 22 January 2013 in Isla de la Luna, Bolivia (16°2'41.24"S, 69°4'8.78"W), a female individual was found dead at 7 m. A post-mortem analysis was carried out and the remains of bones of the legs were found in the intestines of this individual.

This cannibalistic behavior was observed in both wild populations and captive individuals, a fact suggesting that this is a normal behavior in the species. A captive female frog was observed eating two individuals on two different occasions and in another occasion, one male was observed eating a female frog (Fig. 1b,c). Observations also indicate that cannibalism between individuals of different stages is present in the species, with two observations of adult and juvenile frogs eating tadpoles. Cannibalism by larvae was also recorded with two observations of tadpoles attacking and eating other conspecific larvae that were alive, as well as some juveniles that were sick or dead in the same aquarium (Table 1).

Cannibalism in this genus has been recorded so far in *Telmatobius atacamensis* (Barrionuevo 2015), with an adult female predating a juvenile. Prior to our observations, Pérez (1998) reported the remains of a small anuran in the gut contents of wild *T. culeus*, but the identity of the anuran eaten was not specified. To our knowledge, the present report is the first on conspecific predation of adults in this genus. This behavior could be present in this species, as in *Ceratophrys* and *Lepidobatrachus* (Polis 1981), due to the morphological adaptations and size differences between individuals in the population, making the cannibalism of adult individuals possible. It would be interesting to see if the same behavior happens with other species of the genus where no such size differences exist.

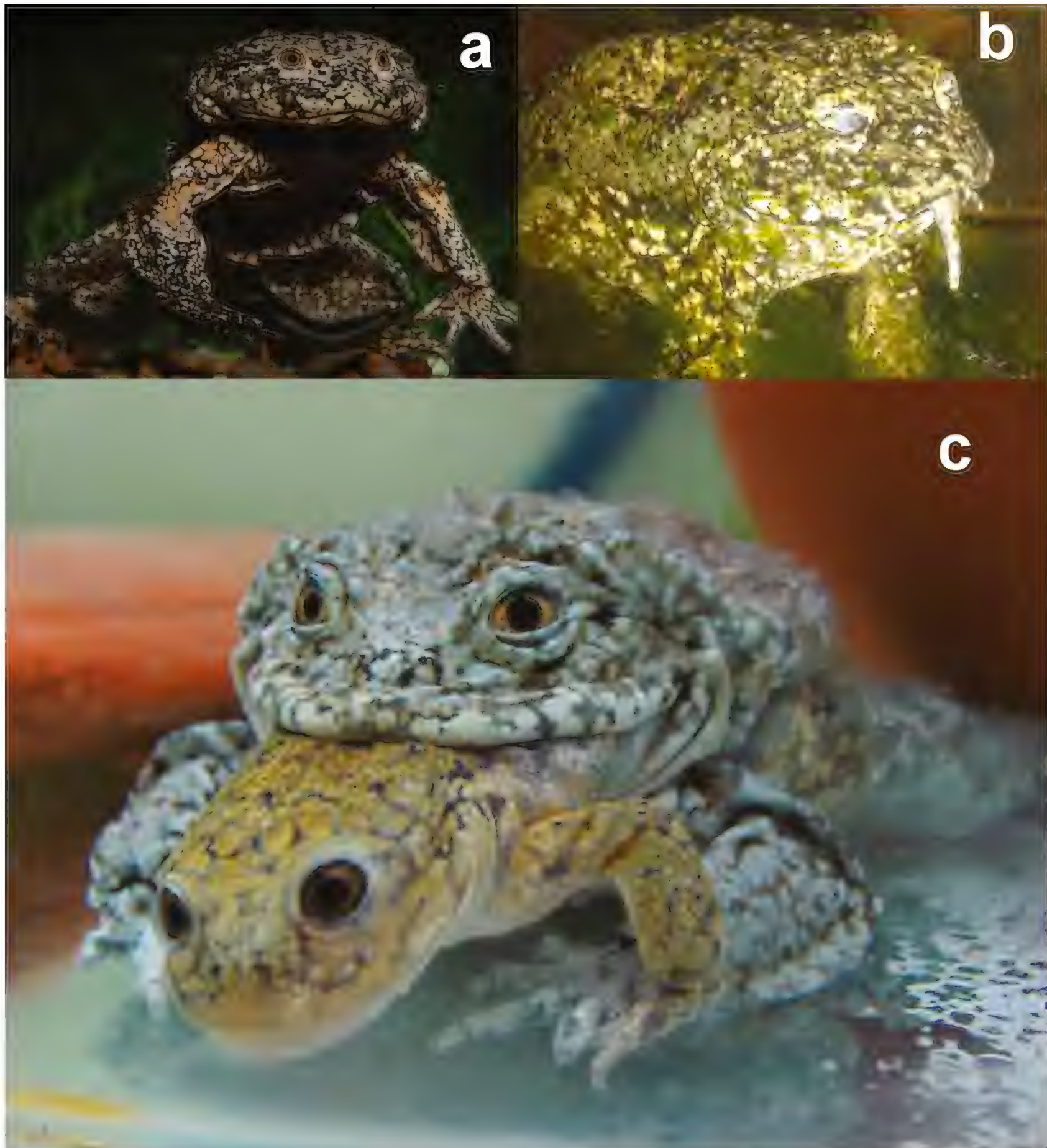


Fig. 1. Individuals of *Telmatobius culeus* eating smaller conspecific frogs: (a) wild male eating a juvenile, (b) female captive frog eating a male adult frog, (c) male captive frog eating a female adult frog. Photos by Arturo Muñoz (a), Patricia Mendoza (b), and Adriana Aguila (c).

Cannibalism may just be opportunistic and occur as a simple by-product of normal predatory behavior (Polis and Myers 1985). Yet, Measey et al. (2015) reported that among 228 anuran species, 77 were known to eat other frogs in different stages. From this last group, cannibalism was identified in 35% of the records. It therefore seems to be a common behavior in anurans, supported by many affirmations (Polis and Myers 1985). The limited number of records of cannibalism in *T. culeus* reported here at least demonstrates that it is present in this species, and in the future more attention should be directed toward seeing if this is a common occurrence in the species. This cannibalistic behavior seems to be associated with taxonomic group, for example taxa such as Ceratophryidae, Hylidae, and Leptodactylidae, all

have elevated levels of anurophagy (Measey et al. 2015). This could be linked with size, where body size is a dominant predictor of anurophagy (Polis 1981; Measey et al. 2015). Since *T. culeus* is considered a large frog (SVL up to 170 mm) and with prominent differences in size between adults, it would make a good candidate for being a cannibalistic species. Adaptations such as a large and wide mouth makes this species prone to predate sizeable prey items, including other anurans and even conspecifics as reported here.

The reasons for cannibalism in a population can be diverse, such as demographic factors where densities are relatively high or where scarce refuge availability increases the chances of encounters (Measey et al. 2015). In some localities, especially in the areas where

Table 1. Details of cannibalism events in wild and captive populations of *Telmatobius culeus*: A = adult, J = juvenile, L = larvae, M = male, F = female, U = undetermined sex.

Locality	Date	Predator characteristics			Prey characteristics		
		Age	Sex	SVL (mm)/weight (g)	Age	Sex	SVL (mm)/weight (g)
Pata Patani	20 January 2009	A	M	112/335	J	U	54/—
Sicuani	4 November 2011	A	F	103/280	A	M	49/—
Isla de La Luna	22 January 2013	A	F	135/302	A	U	N/A
Captive	16 March 2015	A	F	119.4/270	A	M	63.65/28
Captive	16 September 2016	A	F	119.4/270	A	F	62.34/44
Captive	3 January 2017	A	M	91.10/95	A	F	57.18/21
Captive	14 March 2013	A	F	95.6/—	L	U	N/A
Captive	1 May 2013	J	N/A	N/A	L	U	N/A
Captive	4 July 2017	L	N/A	N/A	L	U	N/A
Captive	4 July 2017	L	N/A	N/A	L	U	N/A

cannibalism is reported here, densities of *T. culeus* were relatively high, facilitating the likelihood of individuals finding other frogs that could be eaten. In captive conditions, even with sufficient refuges, cannibalistic events occurred, probably because the chances of encounters were relatively high. Another factor that increases the probability of cannibalism is the similarity of habits that predators and prey share, facilitating their encounters (Measey et al. 2015). In *T. culeus*, as a fully aquatic frog with its entire life cycle under the water, encounters between individuals would be expected to be high and make it easy for large individuals to find tadpoles, juveniles, and even small adults that could be considered as prey.

Heterocannibalism is when there is no genetic relation between a cannibal and its victim (Okuda 2000). There are different reports of its occurrence in aquatic species, such as *Xenopus* and *Pipa*, that are often present in water bodies together with conspecific eggs and tadpoles; and at times when these stages are abundant, they are known to make up a large proportion of the prey eaten (Measey 1998). Here we include two reports of heterocannibalism in captivity in *T. culeus*, but nothing is known about its occurrence in wild populations. Yet, the fact that individuals share the same habitat suggests this kind of cannibalism may also occur in the wild. Similar to *Telmatobius*, *Xenopus* and *Pipa* species lack or have a reduced tongue and rely on suction for most small prey items. They are also able to take large targets through jaw prehension and even the forelimbs are involved in the ingestion of large prey items (Barrionuevo 2016). These are some of the adaptations that *T. culeus*, a fully aquatic species, could use to capture the prey, particularly using the two latter strategies for large prey individuals.

Predating large individuals could be beneficial considering the nutrient intake, but it could also imply costs that result in excessive handling time, as well as a risk of injury to the predator (Wyatt and Forsy 2004). In one of the cases reported here where prey (57.18 mm) and predator (91.1 mm) were of comparable size, the

time that the individual spent trying to ingest the prey was more than 37 hours, involving some costs with the risk of injury during the ingestion and rendering the predatory frog in a vulnerable position against possible predators.

The number of reports on cannibalistic females tends to be higher than for males in the animal kingdom overall (Polis 1981). Despite the low number of observations here, the ratio of four females against two males agrees with that tendency. The great nutritive benefit of such large prey will evidently support the increased nutritional needs of females during the breeding season. Conspecific individuals could thus be a good source of nutrients that makes cannibalism a good option. Even if it is not a common behavior in this species, cannibalism could provide high nutritive value similar to the situation in small fish such as *Orestias*—which are more difficult to catch due to their speed when swimming, when compared with another conspecific prey. Still, males could also benefit from cannibalism, since it allows them to store energy and nutrients for the breeding season. In that regard, it is worth noting that the energy costs for searching and protecting a territory, calling for females, fights, amplexus, and parental care are very high, whereas the males do not eat during this period.

Although cannibalistic polyphenism is known to be present in different species among amphibians (Crump 1983), no information was previously available for *T. culeus*. This species is known to have a great phenotypic variation, to the extent that some have considered there to be different taxa under this name even in the same locality (Benavides et al. 2002). Further studies on this topic would be interesting because all of the cannibalistic frogs reported in this study had the phenotypic features previously ascribed to *Telmatobius albiventris* (now considered *T. culeus*), i.e., all of them were of large size, with wide and big mouths, more robust body and more shaggy skin as seen in Fig. 1. More thorough studies on this topic comparing predator and prey individuals could give us additional information on the likelihood of

cannibalistic polyphenism in this species.

Jiménez and De la Riva (2017) pointed out that cannibalistic lizards predated on large individuals in Andean environments try to optimize digestion by basking to raise their temperature. In the case of *T. culeus*, the species has been reported to bask at hours of higher solar radiation (Muñoz-Saravia et al. 2018), and benefitting digestion could be one of the reasons why these frogs bask. These findings open several questions about the behavior and adaptations of this unique species to some of the extreme conditions found in Titicaca Lake. To determine whether the species really has a cannibalistic behavior or if these are just sporadic observations, could give us more insight about the foraging strategies of the species and the importance of this behavior in the nutrition of the species.

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Literature Cited

- Aguilar C, Valencia N. 2009. Relaciones filogenéticas entre telmatobiinidos (Anura, Ceratophryidae, Telmatobiinae) de los Andes centrales basado en la morfología de los estados larval y adultos. *Revista Peruana de Biología* 16(1): 43–50.
- Barrionuevo J. 2015. *Telmatobius atacamensis* (Atacama Water Frog). Cannibalism. *Herpetological Review* 46: 421.
- Barrionuevo JS. 2016. Independent evolution of suction feeding in Neobatrachia: feeding mechanisms in two species of *Telmatobius* (Anura: Telmatobiidae). *The Anatomical Record* 299: 181–196.
- Benavides AE, Ortiz JC, Sites Jr JW. 2002. Species boundaries among the *Telmatobius* (Anura: Leptodactylidae) of the Lake Titicaca basin: allozyme and morphological evidence. *Herpetologica* 58: 31–55.
- Benavides E. 2005. The *Telmatobius* species complex in Lake Titicaca: applying phylogeographic and coalescent approaches to evolutionary studies of highly polymorphic Andean frogs. Pp. 167–185 In: *Studies on Andean Frogs of the Genera Telmatobius and Batrachophrynus* (Anura: Leptodactylidae). Monografías de Herpetología 7. Editors, Lavilla EO, De la Riva I. Asociación Herpetológica Española, Valencia, Spain. 349 p.
- Cochran DM. 1955. Frogs of southeastern Brazil. *Bulletin of the United States National Museum* 206: 1–423.
- Crump ML. 1983. Opportunistic cannibalism by amphibian larvae in temporary aquatic environments. *The American Naturalist* 121: 281–289.
- Crump ML. 1992. Cannibalism in amphibians. Pp. 256–276 In: *Cannibalism: Ecology and Evolution among Diverse Taxa*. Editors, Elgar MA, Crespi BJ. Oxford University Press, Oxford, United Kingdom. 361 p.
- De La Riva I. 2005. Bolivian frogs of the genus *Telmatobius*: synopsis, taxonomic comments, and description of a new species. Pp. 65–101 In: *Studies on Andean Frogs of the Genera Telmatobius and Batrachophrynus* (Anura: Leptodactylidae). Monografías de Herpetología 7. Editors, Lavilla EO, De la Riva I. Asociación Herpetológica Española, Valencia, Spain. 349 p.
- De la Riva I, García-Paris M, Parra-Olea G. 2010. Systematics of Bolivian frogs of the genus *Telmatobius* (Anura, Ceratophryidae) based on mtDNA sequences. *Systematics and Biodiversity* 8: 49–61.
- Duellman WE, Trueb L. 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore, Maryland, USA. 670 p.
- Fontúrbel RE. 2004. Propuesta de producción sostenible e industrialización del *Telmatobius culeus* (Anura, Leptodactylidae): hacia la conservación de especies endémicas que apoyen el desarrollo sostenible en el lago Huiñaimarca (Bolivia). Pp. 32–40 In: *Aportes al Conocimiento del Manejo de Vida Silvestre en Argentina y Bolivia*. Editor, Fontúrbel RE. Publicaciones. Integrales, La Paz, Bolivia. 103 p.
- Fox LR. 1975. Cannibalism in natural populations. *Annual Review of Ecology and Systematics* 6: 87–106.
- Hoffman EA, Pfennig DW. 1999. Proximate causes of cannibalistic polyphenism in larval Tiger Salamanders. *Ecology* 80: 1,076–1,080.
- Hulse AC. 1978. Food habits of the frog *Lepidobatrachus llanensis* (Amphibia, Anura, Leptodactylidae) in Argentina. *Journal of Herpetology* 12: 258–260.
- Jiménez-Robles O, De la Riva I. 2017. Cannibalism in the Andean lizard *Liolaemus orientalis*. *Studies on Neotropical Fauna and Environment* 52: 244–247.
- Kaplan RH, Sherman PW. 1980. Intraspecific oophagy in California newts. *Journal of Herpetology* 14(2): 183–185.
- Measey GJ. 1998. Diet of feral *Xenopus laevis* (Daudin) in South Wales, United Kingdom. *Journal of Zoology* 246(3): 287–298.
- Measey GJ, Vimercati G, Villiers FA, De Mokhatla MM, Davies SJ, Edwards S, Altwegg R. 2015. Frog eat frog: exploring variables influencing anurophagy. *PeerJ* 3: e1204.
- Muñoz-Saravia A, Callapa G, Janssens G. 2018. Temperature exposure and possible thermoregulation strategies in the Titicaca Water Frog, *Telmatobius culeus*, a fully aquatic frog of the High Andes. *Endangered Species Research* 37: 91–103.
- Okuda N. 2000. Interspecific differences in male

cannibalistic behavior between two sympatric cardinalfishes (Pisces: Apogonidae). *Journal of Ethology* 18(1): 5–10.

Pérez ME. 1998. Dieta y ciclo gametogénico anual de *Telmatobius culeus* (Anura, Leptodactylidae) en el Lago Titicaca (Huiñaimarca). Tesis de Licenciatura en Biología, Departamento de Biología, Universidad Mayor de San Andrés, La Paz, Bolivia. 140 p.

Polis GA. 1981. The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics* 12(1): 225–251.

Polis GA, Myers CA. 1985. A survey of intraspecific predation among reptiles and amphibians. *Journal of*

Herpetology 19: 99–107.

Toledo LF, Ribeiro RS, Haddad CFB. 2007. Anurans as prey: an exploratory analysis and size relationships between predators and their prey. *Journal of Zoology* 271(2): 170–177.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20(1): 249–278.

Wyatt JL, Forsy EA. 2004. Conservation implications of predation by Cuban treefrogs (*Osteopilus septentrionalis*) on native hylids in Florida. *Southeastern Naturalist* 3(4): 695–700.



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Feeding habits of the threatened aquatic Andean frog *Telmatobius rubigo* (Anura: Telmatobiidae)

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Abstract.—The aquatic Andean frogs of the genus *Telmatobius* have evolved closely with the aquatic ecosystems of the Andes of South America. The Laguna de Los Pozuelos' Rusted Frog (*Telmatobius rubigo*) is a Threatened and endemic species of the Central Andean Puna ecoregion in Argentina. This species has a specialized feeding mechanism which relies on the inertial suction of prey, but our knowledge about its natural history is still incomplete. This study examined the feeding habits of *T. rubigo* by the stomach flushing technique. The relevance of the registered prey items was assessed using the dietary importance value index, and the relationship between frog body size and prey volume was determined. In total, 189 prey items were identified in 29 stomach content samples, reaching a representative number of diet samples for this species. *Telmatobius rubigo* had a fully aquatic diet, with a clear predominance of adult aquatic coleopterans, immature stages of benthic insects, and crustaceans; and a high incidence of non-nutritive elements (sand and vegetation debris) was also found in the stomach contents. The results indicate that the species exhibits generalist feeding habits, and the volume of consumed prey items is positively related to the body size of the frogs. We suggest that the species develops mainly an active search mode of their benthic prey. This study represents one of the most complete dietary records for a *Telmatobius* species, and helps us to understand the ecology of this species in the extreme desert environment of the high Andes Puna. These results can contribute to the conservation efforts being made for *Telmatobius* species.

Keywords. Aquatic prey, diet, Puna, stomach flushing, suction feeding, trophic niche

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Introduction

Telmatobius is a genus of anuran amphibians that has closely evolved with the aquatic ecosystems of the Andes of South America; and unlike other amphibian taxa, these aquatic Andean frogs are actually more diverse at high altitudes (Barrionuevo 2017). The aquatic life habit in such demanding high-altitude environments poses a series of biological and physiological challenges for these frogs (Lavilla and De la Riva 2005). In this regard, there is only fragmentary knowledge about the feeding habits of the aquatic Andean frogs; and what little is known is mostly based on occasional observations made on a limited number of individuals (Garman 1876; Allen 1922; Barrionuevo 2015, 2016; Cuevas and Formas 2002; Formas et al. 1999; Wiens 1993). Few studies have assessed the trophic ecology of *Telmatobius* species with a representative number of individuals that is sufficient to show the predominance of invertebrate aquatic prey in

their diets (Lavilla 1984; Lobos et al. 2016; Valencia et al. 1982; Watson et al. 2017).

In Argentina, the species of genus *Telmatobius* are significantly threatened, with the main causes of decline being habitat alteration, the introduction of exotic predatory fishes, chytrid fungus infection, and the indirect consequences of extreme climate events (Barrionuevo and Mangione 2006; Barrionuevo and Ponssa 2008; IUCN 2020; Vaira et al. 2012). Despite the increasing level of concern regarding the conservation of the aquatic Andean frogs, there is very little information about the natural history of the 15 species registered in the country (Duré et al. 2018; Vaira et al. 2012).

The Laguna de Los Pozuelos' Rusted Frog (*Telmatobius rubigo* Barrionuevo and Baldo, 2009) is the most recently described species of genus *Telmatobius* in Argentina. This species is endemic to the Central Andean Puna ecoregion of Jujuy province in Argentina, particularly in the arid river systems of the Laguna

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Fig. 1. Adult male of *Telmatobius rubigo* in its natural habitat in the locality of Santa Catalina, Jujuy province, Argentina. Photo by Mauricio Sebastián Akmentins.

de Los Pozuelos basin (Barrionuevo and Abdala 2018; Barrionuevo and Baldo 2009). This fully aquatic frog has a unique feeding behavior among anurans, using a specialized feeding mechanism of inertial suction to capture their prey (Barrionuevo 2016). Beyond this singular prey capture mechanism, the knowledge about the trophic ecology of this species remains incomplete.

This study analyzed the feeding habits of the Laguna de Los Pozuelos' Rusted Frog in the desert Puna environment of Jujuy province, Argentina. Due to the combination of a strictly aquatic life habit and the inertial suction feeding mechanism, we expected a predominance of aquatic items in the diet of this species. Determining the composition of prey can provide valuable biological information to better understand the ecology of this threatened aquatic Andean frog.

Materials and Methods

The study was conducted in three localities of occurrence of *Telmatobius rubigo* in Jujuy province, Argentina (Barrionuevo and Abdala 2018): Queta, in the southern distributional range (22°43'7.88"S, 65°58'19.71"W; 3,548 m asl); Casa Colorada, in the western distributional range (22°22'8.9"S, 66°13'29.7"W; 4,333 m asl); and Santa Catalina, in the northern distributional range, near the type locality of the species (21°56'58.2"S, 66°02'21.6"W; 3,802 m asl). These localities are in the Central Andean Puna ecoregion (Dinerstein et al. 1995). The climate is typical of high-altitude desert, being cold and dry with large daily thermal fluctuations. Precipitation events are scarce, occurring as snow and hail in the winter and rain in summer (Barrionuevo and Baldo 2008).

The frogs were located in the rivers through an active search by visual encounter (Crump and Scott 1994), during January and March 2020 (Fig. 1). The frogs were captured manually, and the stomach contents were obtained *in situ* by the modified technique of stomach flushing (Legler and Sullivan 1979; Solé et al. 2005), which avoids mortality of the frogs. The stomach contents were individually preserved with 70% ethanol in 1.5 ml polypropylene tubes for subsequent analysis. For each frog, the sex was recorded based on secondary sexual characters, such as nuptial pads and keratinized spicules on the chest (Barrionuevo and Baldo 2009). The size of each frog was measured as the Snout-Vent Length (SVL) with a digital dial caliper to the nearest 0.1 mm (Mitutoyo Absolute Digimatic, Kawasaki, Japan) and each frog was weighed with a portable digital scale to the nearest 0.1 g (OHAUS, Parsippany, New Jersey, USA). After diet samples and measurements were taken, the frogs were released at the capture site.

The stomach contents were analyzed under a stereomicroscope, and prey were identified to the level of subclass for Annelida, and to the level of order or family for Arthropoda. For each item (prey category), the number (N), volume (V), and occurrences (F) were calculated as both absolute and percentage values. The volume for intact prey items was estimated according to the formula used by Dunham (1983) for a prolate spheroid: $V = \frac{4}{3} \pi \times (\text{prey length}/2) \times (\text{prey width}/2)^2$.

The representativeness of the diet sample was evaluated by constructing a coverage-based (species richness) rarefaction curve for incidence data (Chao and Jost 2012), using iNEXT package, version 2.0.5 (Chao et al. 2016) in the program R (R Core Team 2017).

The dietary importance value index for pooled stomach samples was calculated to determine the importance of each prey item according to the formula described by Biavati et al. (2004): $I_p = (N\% + V\% + F\%)/3$, where $N\%$ is numeric percentage, $V\%$ is volumetric percentage, and $F\%$ is occurrence percentage. Intraspecific differences in diet composition were explored by calculating the I_p values for pooled stomach samples classified by sex (females and males).

The trophic niche breadth, for the species and by sex, were calculated using Levin's standardized index (Krebs 1989):

$$B = \frac{1}{(n-1)} \left[\frac{1}{(\sum_i^n P_i^2)} - 1 \right]$$

where n is the total number of prey items, and P_i is the proportion of prey item i in the stomach contents. Breadth niche values range from 0 to 1, and were arbitrarily set here as high (> 0.6), intermediate (0.4 to 0.6), or low (< 0.4), according to Novakowski et al. (2008).

The degree of diet overlap between females and males was calculated using the Morisita-Horn Index (Horn 1966):

$$\hat{C}\lambda = \frac{2 \sum_i^n P_{ij} P_{ik}}{\sum_i^n P_{ij}^2 + \sum_i^n P_{ik}^2}$$

where n is the total number of prey items, P_{ij} is the proportion of the prey item i consumed by females, and P_{ik} is the proportion of the prey item i consumed by males. Values greater than 0.60 were considered to represent a significant diet overlap (Zaret and Rand 1971).

The biometric measures of females and males were compared using a two-sample t -test to compare the SVL (normal distribution), and a Mann-Whitney U -test to compare weight (non-normal distribution). A linear regression analysis was used to test the relationship between the frog size (SVL) and the log-transformed mean volume of the consumed prey (Hodgkinson and Hero 2003). For all analyses, $p < 0.05$ was considered to represent a statistically significant difference.

Results

Thirty-one diet samples were obtained from 12 females, 18 males, and one indeterminate individual. Females had a body size of 51.9 ± 6.5 mm (mean SVL \pm SD) and weight of 12.2 ± 5.3 g (mean \pm SD). Males had a body size of 48.4 ± 6.7 mm (mean SVL \pm SD) and weighed 10.8 ± 4.4 g (mean \pm SD). For the individual of undetermined sex, the SVL was 40 mm and the body mass was 5.8 g. No significant differences were found between the sexes in the SVL ($t = 1.44$; $p = 0.16$) or the body mass (Mann-Whitney: $U = 95$; $p = 0.539$).

A total of 189 prey items were identified in 29 of the 31 stomach content samples, with a mean number of prey items per stomach of 6.5 ± 6.4 (mean \pm SD).

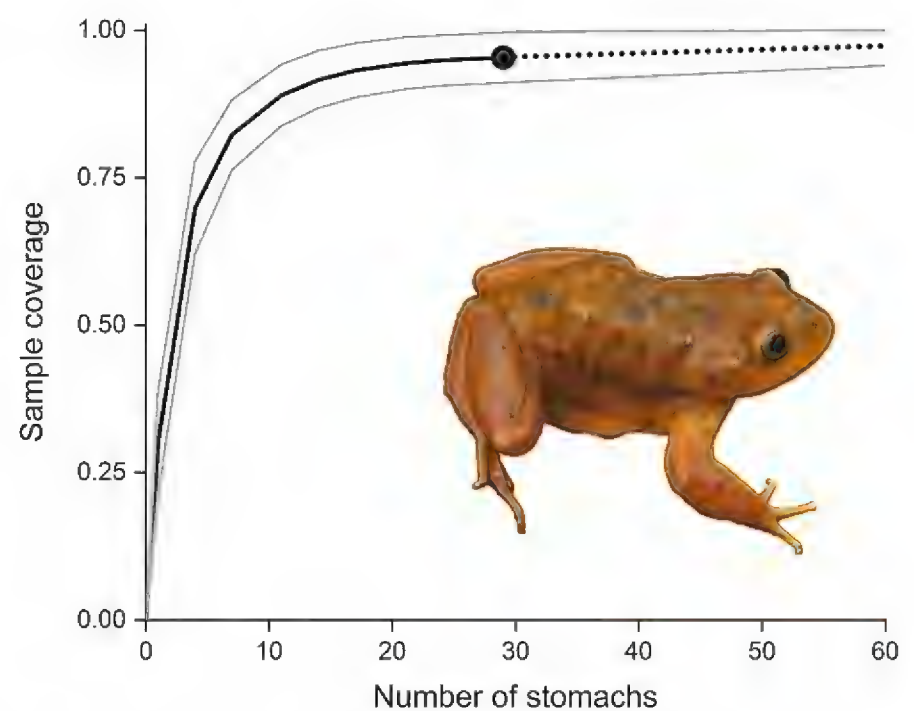


Fig. 2. Coverage-based rarefaction (solid line) and extrapolation (dotted line) curves for prey sample completeness (Hill numbers of order $q = 0$) of the analyzed stomachs of *Telmatobius rubigo*. The 95% confidence interval boundaries (gray lines) were calculated based on 200 bootstrap replicates.

The coverage-based rarefaction curve showed that the sampling effort was appropriate and reached 95.4% of completeness for prey richness (Fig. 2).

The trophic niche of *T. rubigo* was found to be based on invertebrates, with prey representing a wide range of taxa and a greater diversity of insects. The most important prey were adults of aquatic coleopteran families Dytiscidae and Elmidae, and the remainder of the diet was mainly composed of slow-moving benthic prey with a clear predominance of larvae of Coleoptera and Diptera. Crustaceans also were a relevant food item, dominated by *Hyalella* sp. shrimps and diminutive ostracods. The only relevant allochthonous prey items found in the diet of *T. rubigo* were earthworms. Vegetal debris and/or sand were registered in more than half of the stomach contents analyzed. *Telmatobius rubigo* has an intermediate niche

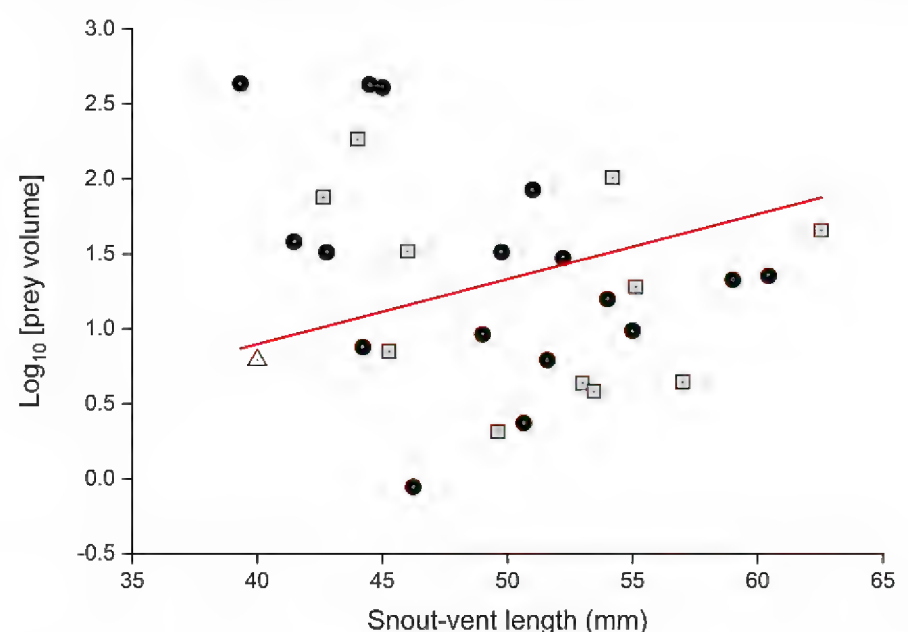


Fig. 3. Relationship between Snout-Vent Length (SVL) of *Telmatobius rubigo* and log-transformed mean volume of the consumed prey. The white triangle represents the indeterminate individual, grey squares represent female individuals, and black circles represent male individuals. The red line represents the linear fit estimated by the regression analysis considering all individuals.

Table 1. Summary of the identified prey items, with the absolute values and percentages of number (N), volume (V, in mm³), frequency of occurrence (F), and dietary importance value index (*Ip*) of the principal prey items consumed by *Telmatobius rubigo*. The development stages of the insect prey items are specified in parentheses. Categories with *Ip*-values above 10% are in bold. In the last row, Levin's standardized index of trophic niche breadth is given for *T. rubigo*.

Prey taxa	N (%)	V (%)	F (%)	<i>Ip</i>
ANNELIDA				
Hirudinea	1 (0.5)	10.6 (0.3)	1 (3.4)	1.4
Oligochaeta*	8 (4.2)	1339.5 (36.7)	4 (13.8)	18.2
ARTHROPODA				
Crustacea				
Amphipoda	30 (15.9)	265.7 (7.3)	13 (44.8)	22.7
Hyalellidae				
Ostracoda	20 (10.6)	10.9 (0.3)	6 (20.7)	10.5
Isopoda*	6 (3.2)	75.4 (2.1)	1 (3.4)	2.9
Hexapoda				
Coleoptera (larvae)	25 (13.2)	157 (4.3)	13 (44.8)	20.8
Elmidae				
Dytiscidae				
Coleoptera (adult)	45 (23.8)	663.9 (18.2)	15 (51.7)	31.2
Elmidae				
Dytiscidae				
Diptera (larvae)	25 (13.2)	44.5 (1.2)	10 (34.5)	16.3
Chironomidae				
Muscidae				
Tabanidae				
Syrphidae				
Diptera (adult)	4 (2.1)	8.5 (0.2)	4 (13.8)	5.4
Ephemeroptera (nymph)	9 (4.8)	62.7 (1.7)	6 (20.7)	9.1
Hemiptera (adult)	7 (3.7)	118.1 (3.2)	3 (10.3)	5.8
Notonectidae				
Hymenoptera (adult)*	5 (2.6)	18 (0.5)	5 (17.2)	6.8
Formicidae				
Lepidoptera (larvae)*	1 (0.5)	147.3 (4.0)	1 (3.4)	2.7
Odonata (nymph)	2 (1.1)	617.0 (16.9)	2 (6.9)	8.3
Odonata (adult)*	1 (0.5)	110.4 (3.0)	1 (3.4)	2.3
Vegetal debris**			19 (65.5)	
Sand**			17 (58.6)	
Levin's standardized index	0.45			

*Allochthonous prey items; **only considering the frequency of occurrence in the stomach contents.

breadth, and Table 1 shows a summary of the quantitative analysis of the diet for the species.

Analyzing the prey consumption data by sex revealed differences in the prey importance between females and males, with females showing a wider trophic niche than males. There was a significant diet overlap among sexes. Table 2 shows a summary of the quantitative analysis of diet for each sex. A significant positive relationship ($R^2 = 0.382$, $p < 0.05$) was found between the body size of the frogs and the log-transformed mean volume of consumed prey (Fig. 3).

Discussion

The registered prey items in the stomach contents of the Laguna de Los Pozuelos' Rusted Frog showed a clear predominance of small slow-moving and gregarious benthic prey, confirming the hypothesis of a fully aquatic diet which coincides with the strictly aquatic life habits of this species.

As found here in the diet of *Telmatobius rubigo*, a predominance of adult insects also was observed in *T. hauthali* (Lavilla 1984). In addition, several studies have

Table 2. Dietary importance value index (*Ip*) of the prey items consumed by females and males of *Telmatobius rubigo*. The development stages of the insect prey item are specified in parentheses. Categories with *Ip*-values above 10% are in bold. The two last rows show Levin’s standardized index of trophic niche breadth for each sex of *T. rubigo*, and the Morisita-Horn index of dietary overlap between the sexes.

Prey taxa	<i>Ip</i> females	<i>Ip</i> males
Hirudinea	—	0.4
Oligochaeta*	3.6	20.6
Amphipoda	16.5	8.2
Ostracoda	7.1	5.1
Isopoda*	—	2.7
Coleoptera (larva)	12.2	7.6
Coleoptera (adult)	19.3	12.3
Diptera (larva)	3.6	6.0
Diptera (adult)	10.7	0.3
Ephemeroptera (nymph)	12.2	1.5
Hemiptera (adult)	13.8	—
Hymenoptera (adult)*	10.7	0.7
Lepidoptera (larva)*	3.6	—
Odonata (nymph)	3.6	0.3
Odonata (adult)*	—	1.9
Levin’s standardized index	0.66	0.51
Morisita-Horn index	0.71	

*Allochthonous prey items.

shown that the immature stages of benthic insects are the most common items in the trophic niche of other aquatic Andean frogs (Lavilla 1984; Lobos et al. 2016; Valencia 1982; Watson et al. 2017). The amphipod shrimps also are a representative prey in the diet of various *Telmatobius* frogs (Allen 1922; Lobos et al. 2016; Valencia 1982; Watson et al. 2017), and other species of strictly aquatic anurans of Argentina (Cuello et al. 2006; Velasco et al. 2019). The presence of allochthonous prey, such as earthworms, indicates that they were most likely consumed underwater when they accidentally fell from the riverbanks.

The common presence of non-nutritive elements, mainly vegetal debris and sand, in the stomachs of *T. rubigo* is evidence of the strategy of foraging in the benthos of rivers and is related to the suction force of the feeding mechanism used by these frogs for capturing their prey (Barrionuevo 2016). A similar high incidence of non-nutritive items in the stomach contents was also reported in Chilean species of the *Telmatobius marmoratus* group (Valencia 1982), suggesting a shared foraging tactic in this species group (Barrionuevo 2017).

Overall, these results show that *T. rubigo* exhibits generalist and opportunistic feeding habits, as has been noted in other species of *Telmatobius* (Lavilla 1984; Valencia 1982). We suggest that *T. rubigo* performs a mainly active search of their prey. Despite the differences in the importance of consumed prey and the width of the

trophic niche between females and males of *T. rubigo*, we suggest there is no trophic niche segregation due to the absence of sexual dimorphism in biometric measures and the high diet overlap between the sexes.

Telmatobius rubigo was found in high densities in some parts of the surveyed river systems, occurring in sympatry with two other anuran species, *Pleurodema cinereum* and *Rhinella spinulosa*. We did not register any cases of cannibalism or anurophagy in *T. rubigo*, as has been reported in some other *Telmatobius* species (Allen 1922; Barrionuevo 2015; Valencia et al. 1982; Wiens 1993). The insight of trophic niche segregation in *T. rubigo* indicated here, due to the relationship between the mean volume of consumed prey with the frog size, may be a mechanism to avoid competition and cannibalism. However, a more in-depth analysis including seasonal changes in the diet and prey availability/prey selection will be necessary to fully understand the trophic ecology of Laguna de Los Pozuelos’ Rusted Frog.

Conclusions

The results of this study have direct contributions for understanding the ecology of Laguna de Los Pozuelos’ Rusted Frog (*Telmatobius rubigo*), as well as for its conservation. *Telmatobius rubigo* is threatened by direct and indirect consequences of human activities (IUCN 2020; Vaira et al. 2012). Among the direct threats that we observed during our fieldwork are the poor water management for human and animal consumption, the introduction of exotic predatory fishes (salmonids), inappropriate management of solid waste, liquid effluents from human settlements, and mining leachate pollution. All these threats affect not only the aquatic Andean frogs but also the populations of their aquatic invertebrate prey (Valdovinos et al. 2007; Van Damme et al. 2008; Vimos et al. 2015).

As far as we know, this study represents one of the most complete dietary records for an aquatic Andean frog of the genus *Telmatobius*, due to the numbers of surveyed localities and sampled individuals. The results obtained in this study can be used as a guide for the *ex situ* conservation efforts being made for *Telmatobius* species, particularly for improving the food provided to these frogs in captive breeding programs.

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Literature Cited

Allen WR. 1922. Notes on the Andean frog, *Telmatobius culeus* (Garman). *Copeia* 108: 52–54.

- Barrionuevo JS. 2015. *Telmatobius atacamensis*. Cannibalism. *Herpetological Review* 46(3): 420–421.
- Barrionuevo JS. 2016. Independent evolution of suction feeding in Neobatrachia: feeding mechanisms in two species of *Telmatobius* (Anura: Telmatobiidae). *The Anatomical Record* 299: 181–196.
- Barrionuevo JS. 2017. Frogs at the summits: phylogeny of the Andean frogs of the genus *Telmatobius* (Anura, Telmatobiidae) based on phenotypic characters. *Cladistics* 33(1): 41–68.
- Barrionuevo JS, Abdala CS. 2018. Herpetofauna de la Puna Argentina: una síntesis. Pp. 209–228 In: *La Puna Argentina: Naturaleza y Cultura. Serie Conservación de la Naturaleza* 24. Editors, Grau HR, Babot MJ, Izquierdo AE, Grau A. Fundación Miguel Lillo, Tucumán, Argentina. 499 p.
- Barrionuevo JS, Baldo D. 2009. A new species of *Telmatobius* (Anura, Ceratophryidae) from northern Jujuy Province, Argentina. *Zootaxa* 2303: 1–20.
- Barrionuevo JS, Mangione S. 2006. Chytridiomycosis in two species of *Telmatobius* (Anura; Leptodactylidae) from Argentina. *Diseases of Aquatic Organisms* 73: 171–174.
- Barrionuevo JS, Ponssa ML. 2008. Decline of three species of the genus *Telmatobius* (Anura: Leptodactylidae) from Tucumán Province, Argentina. *Herpetologica* 64(1): 47–62.
- Biavati GM, Wiederhecker HC, Colli GR. 2004. Diet of *Epipedobates flavopictus* (Anura: Dendrobatidae) in a Neotropical savanna. *Journal of Herpetology* 38(4): 510–518.
- Chao A, Jost L. 2012. Coverage based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93(12): 2,533–2,547.
- Chao A, Ma KH, Hsieh TC. 2016. iNEXT (iNterpolation and EXTrapolation) online: software for interpolation and extrapolation of species diversity. Program and user's guide. Available: http://chao.stat.nthu.edu.tw/wordpress/software_download [Accessed: 9 June 2020].
- Crump ML, Scott NJ. 1994. Standard techniques for inventory and monitoring. Pp. 76–141 In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Editors, Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 364 p.
- Cuello ME, Bello MT, Kun M, Úbeda CA. 2006. Feeding habits and their implications for the conservation of the Endangered semiaquatic frog, *Atelognathus patagonicus* (Anura, Neobatrachia) in a northwestern Patagonian pond. *Phyllomedusa* 5(1): 67–76.
- Cuevas CC, Formas JR. 2002. *Telmatobius philippii*, una nueva especie de rana acuática de Ollagüe, norte de Chile (Leptodactylidae). *Revista Chilena de Historia Natural* 75: 245–258.
- Dinerstein E, Olson DM, Graham DJ, Webster AL, Primack RB, Bookbinder M, Ledec G. 1995. *Una Evaluación del Estado de las Eco-regiones Terrestres de América Latina y el Caribe*. Banco Mundial, Washington, DC, USA. 135 p.
- Dunham AE. 1983. Realized niche overlap, resource abundance, and intensity of interspecific competition. Pp. 261–280 In: *Lizard Ecology: Studies of a Model Organism*. Editors, Huey RB, Pianka ER, Schoener TW. Harvard University Press, Cambridge, Massachusetts, USA. 507 p.
- Durán M, Úbeda C, Kacoleris FP, Schaefer E, Pereyra LC, Acosta JC, Martínez TA, Rodríguez Muñoz MJ, Akmentins MS, et al. 2018. Componente 4. Evaluación y categorización del estado de conservación. Pp. 15–19 In: *Plan de Acción para la Conservación de los Anfibios de la República Argentina*. Cuadernos de Herpetología, Volume 32, Suplemento 1. Editors, Vaira M, Akmentins MS, Lavilla EO. Asociación Herpetológica Argentina, Jujuy, Argentina. 56 p.
- Formas JR, Northland I, Capetillo J, Nuñez JJ, Cuevas CC, Brieva LM. 1999. *Telmatobius dankoi*, una nueva especie de rana acuática del norte de Chile (Leptodactylidae). *Revista Chilena de Historia Natural* 72: 427–445.
- Garman S. 1876. Exploration of Lake Titicaca. I. Fishes and reptiles. *Bulletin of the Museum of Comparative Zoölogy* 3(11): 273–278.
- Hodgkison S, Hero JM. 2003. Seasonal, sexual, and ontogenetic variations in the diet of the “declining” frogs *Litoria nannotis*, *Litoria rheocola*, and *Nyctimystes dayi*. *Wildlife Research* 30(4): 345–354.
- Horn HS. 1966. Measurement of “overlap” in comparative ecological studies. *The American Naturalist* 100(914): 419–424.
- IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. IUCN, Gland, Switzerland. Available: <https://www.iucnredlist.org> [Accessed: 15 June 2020].
- Krebs CJ. 1989. *Ecological Methodology*. Harper and Row, New York, New York, USA. 654 p.
- Lavilla EO. 1984. Redescubrimiento de *Telmatobius hauthali* Koslowsky, 1985, y descripción de su larva. *Acta Zoologica Lilloana* 38(1): 51–57.
- Lavilla EO, De la Riva I. 2005. Estudios sobre las ranas andinas de los géneros *Telmatobius* y *Batrachophrymus* (Anura: Leptodactylidae). *Monografías de Herpetología* 7: 65–101.
- Legler JM, Sullivan LJ. 1979. The application of stomach-flushing to lizards and anurans. *Herpetologica* 35(2): 107–110.
- Lobos G, Rebolledo N, Charrier A, Rojas O. 2016. Natural history notes of *Telmatobius dankoi* (Anura, Telmatobiidae), a Critically Endangered species from northern Chile. *Studies on Neotropical Fauna and Environment* 51: 152–157.
- Novakowski GC, Hahn NS, Fugli R. 2008. Diet seasonality and food overlap of the fish assemblage in a pantanal

- pond. *Neotropical Ichthyology* 6(4): 567–576.
- R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <https://www.R-project.org/> [Accessed: 5 June 2020].
- Solé M, Beckmann O, Pelz B, Kwet A, Engels W. 2005. Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. *Studies on Neotropical Fauna and Environment* 40(1): 23–28.
- Vaira M, Akmentins M, Attademo M, Baldo D, Barrasso D, Barrionuevo S, Basso N, Blotto B, Cairo S, Cajade R, et al. 2012. Categorización del estado de conservación de los anfibios de La República Argentina. *Cuadernos de Herpetología* 26(Suppl 1): 131–159.
- Valdovinos C, Moya C, Olmos V, Parra O, Karrasch B, Buettner O. 2007. The importance of water-level fluctuation for the conservation of shallow water benthic macroinvertebrates: an example in the Andean zone of Chile. *Biodiversity and Conservation* 16: 3,095–3,109.
- Valencia J, Veloso A, Sallaberry M. 1982. Nicho trófico de las especies de los herpetozoos del transecto Arica-Chungará. Pp. 269–289 In: *La Vegetación y Vertebrados Ectotérmicos del Transecto Arica- Lago Chungará. Volume I*. Editors, Veloso A, Bustos E. ROSTLAC, Montevideo, Uruguay. 327 p.
- Van Damme PA, Hamel C, Ayala A, Bervoets L. 2008. Macroinvertebrate community response to acid mine drainage in rivers of the High Andes (Bolivia). *Environmental Pollution* 156(3): 1,061–1,068.
- Velasco MA, Akmentins MS, Kass CA, Kacoliris FP. 2019. Diet of Critically Endangered Valcheta Frog, *Pleurodema somuncurens* (Anura: Leptodactylidae), in the Somuncura Plateau, Patagonia, Argentina. *North-western Journal of Zoology* 15(2): 147–151.
- Vimos DJ, Encalada AC, Ríos-Touma B, Suárez E, Prat N. 2015. Effects of exotic trout on benthic communities in high-Andean tropical streams. *Freshwater Science* 34(2): 770–783.
- Watson AS, Fitzgerald AL, Baldeón OJD. 2017. Diet composition and prey selection of *Telmatobius macrostomus*, the Junín Giant Frog. *Endangered Species Research* 32: 117–121.
- Wiens JJ. 1993. Systematics of the leptodactylid frog genus *Telmatobius* in the Andes of northern Peru. *Occasional Papers of the Museum of Natural History, the University of Kansas* 162: 1–76.
- Zaret TM, Rand AS. 1971. Competition in tropical stream fishes: support for the competitive exclusion principle. *Ecology* 52(2): 336–342.



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A new species of green pit viper of the genus *Trimeresurus* Lacépède, 1804 (Reptilia: Serpentes: Viperidae) from the Nicobar Archipelago, Indian Ocean

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Abstract.—A new species of green pit viper of the genus *Trimeresurus*, in the *T. albolabris* complex, is described from Car Nicobar Island of the Nicobar Archipelago, Indian Ocean. The new species, *Trimeresurus davidi* sp. nov., can be distinguished from all other members of this group by the following characteristics: medium to large body size (277–835 mm SVL); dorsal scales in a series of 21–25:21–23:15–17 rows; nasal partly fused with the first supralabial; 166–179 ventrals, 58–70 subcaudals; one preocular; 2–3 postoculars; 10–12 supralabials; 12–15 infralabials; two internasals usually in contact with each other; 11–14 cephalic scales; verdant green dorsal and ventral color, absence of white ventrolateral stripes along the sides of the body; males with a white supralabial streak, bordered by a reddish tinge above; a pair of white and red stripes along the sides of the tail in both sexes; a reddish brown colored tail and a greenish iris. The new species is endemic to Car Nicobar Island of the Nicobar Archipelago, and should be regarded as an Endangered species owing to its restricted distribution range.

Keywords. Endangered, endemic species, Nicobar Islands, Reptilia, Squamata, *Trimeresurus albolabris* complex

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Introduction

Asian pit vipers of the genus *Trimeresurus* Lacépède, 1804 are currently represented by 50 species ranging from the Western Ghats of peninsular India in the west to the Lesser Sunda Islands in the east (Uetz et al. 2020; Vogel 2008). Among these species, the white-lipped green pit vipers include five species in the *T. albolabris* complex, which constitute nearly 10% of the diversity, and their collective geographic distribution ranges from the Western Himalayan Mountains (for *T. septentrionalis*; Kramer 1977) in the west to the Lesser Sunda Islands (for *T. insularis*; Kramer 1977) in the east (Vogel 2008; Kramer 1977; Mirza et al. 2020; Chen et al. 2020; Uetz et al. 2020). The white-lipped pit vipers are currently classified under the subgenus *Trimeresurus* under the genus *Trimeresurus* (David et al. 2011). Two new members, namely *T. salazar* Mirza, Bhosale, Phansalkar, Sawant, Gowande, Patel, 2020 and *T. caudornatus* Chen, Yu, Vogel, Shi, Song, Tang, Yang,

Ding, Chen, 2020, have recently been added to this group (Mirza et al. 2020; Chen et al. 2020). One member of this species complex, traditionally identified as *Trimeresurus albolabris* Gray, 1842 (Smith 1943; Vijayakumar and David 2006), has been reported from Car Nicobar Island of the Nicobar Archipelago in the past (Smith 1943; Vijayakumar and David 2006; Vogel et al. 2014). Herein, we reassess the systematic status of this insular population of *Trimeresurus* and provide evidence for its specific distinction from *T. albolabris sensu stricto*, thereby describing it as a species new to science.

Materials and Methods

Pit vipers of the genus *Trimeresurus* encountered in the field (on Car Nicobar) were carefully restrained, measured, and scored for morphological characters, followed by their release back into their native habitat. One dead specimen from Chuckchucka Village (9.2179°N, 92.8003°E, 6 m asl), Car Nicobar, was collected and deposited in the collection

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Fig. 1. Holotype (BNHS 3304) of *Trimeresurus davidi* **sp. nov.** Photo by Rahul Khot.

of the Department of Ocean Studies and Marine Biology, Pondicherry University (DOSMB), Port Blair, India. Six specimens of this species available in the collections of the Natural History Museum, London (NHMUK), which were collected during Lord Moyne's expedition to the Nicobar Islands (Smith 1943), were examined for comparison. One additional specimen deposited in the collections of the Bombay Natural History Society (BNHS) was studied and ascribed to this species.

The following characters were recorded: snout-vent length (SVL); tail length (TaL); total length (TL; SVL+TaL); head length, measured from the snout tip to the jaw angle (HL); head width at the level of the eyes (HW); maximum head depth (HD); horizontal diameter of the eye (ED); eye-nostril distance (EN); snout length, from anterior margin of the eye to snout tip (ES); inter-orbital distance, measured dorsally as the distance between the eyes (IO); inter-narial distance, measured as the distance between the nares (IN); dorsal scale-rows near neck, at midbody and near tail (DSR); number of cephalic scales, counted in a horizontal series between the elongated supraoculars (CEP); and ventrals, counted following Dowling (1951). The sex of the specimens was determined by examination for presence or absence of hemipenis by palpating the tail, coupled with the relative tail length, expressed as the ratio of tail length to the total length of the snake (TaL/TL). Individuals with incomplete/regenerated tails (bold values in Table 2) are excluded from the relative tail length range. Geographic coordinates of the localities of the specimen occurrences were recorded with a Garmin GPSMAP 78s and mapped with ARC MAP v. 10.

Museum acronyms for comparative specimens examined (Appendix 1) are as follows: NHMUK: Natural History Museum [formerly the British Museum (Natural History)], London, United Kingdom; CAS: California Academy of Sciences, San Francisco, California, USA; CIB: Chengdu Institute of Biology, Chengdu, People's Republic of China; MHNG: Muséum d'Histoire Naturelle, Ville de Genève, Switzerland; MNHN: Muséum National d'Histoire Naturelle, Paris, France; NMW: Naturhistorisches Museum Wien, Austria; RMNH: Nationaal Natuurhistorisch Museum (Naturalis), Leyden, The Netherlands; NHMB: Naturhistorisches Museum Basel, Switzerland; SMF: Natur-Museum und Forschungs-Institut Senckenberg, Frankfurt-am-Main, Germany; ZMB: Zoologisches Museum für Naturkunde der Humboldt-Universität zu Berlin, Berlin, Germany; ZMH: Zoologisches Museum Hamburg [formerly Zoologisches Institut und Museum], Universität Hamburg, Hamburg, Germany; ZSI: Zoological Survey of India, Kolkata [Calcutta], India.

Systematics

Trimeresurus davidi **sp. nov.** (Figs. 1–2)

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Trimeresurus albolabris – Vijayakumar and David (2006).

Trimeresurus albolabris – Smith (1943) part, Vogel (2008) part, Vogel et al. (2014) part.



Fig. 2. *Trimeresurus davidi* sp. nov. in life from Car Nicobar (top and middle: males, bottom: female).

Holotype. BNHS 3304, an adult female from Chuckchucka Village (9.2161°N, 92.8109°E, 14 m asl), Car Nicobar, collected by a group of Nicobari men (*vide* Vijayakumar and David 2006).

Paratypes. DOSMB 05104, an adult male from Chuckchucka Village, Car Nicobar; NHMUK 1936.7.7.40, NHMUK 1936.7.7.41, NHMUK 1936.7.7.42, (three adult females from ‘Car Nicobar, Nicobar Is.’), NHMUK 1936.7.7.46 an unsexed adult from ‘Car Nicobar, Nicobar Is.’, NHMUK 1936.7.7.47 and NHMUK 1936.7.7.48 (two adult males from ‘Nicobar Is.’ and ‘Andaman Is.’ [doubtful], respectively), collected during Lord Moyne’s expedition to the Nicobar Islands.

Etymology. The specific epithet is a patronym, named in genitive singular case, honoring Patrick David, an eminent reptile taxonomist for his immense contribution to the systematics of Asian pit vipers and, in particular, to the Nicobar snake fauna.

Diagnosis. *Trimeresurus davidi* sp. nov. is an arboreal member of the genus *Trimeresurus* restricted to the Car Nicobar Island of the Nicobar archipelago, characterized by: medium to large sized body (277–835 mm SVL); dorsal scales in a series of 21–25:21–23:15–17 rows; nasal partly fused with the first supralabial; 166–179 ventrals; 46–70 subcaudals; one preocular; 2–3 postoculars;

10–12 supralabials; 12–15 infralabials; two internasals usually in contact with each other; 11–14 cephalic scales; relative tail length (TaL/TL) ranging from 0.143–0.20; dorsal and ventral verdant green in color, lacking white ventrolateral stripes; males with a white supralabial streak, bordered by a reddish tinge above; a pair of white and red stripes along the sides of the tail in both males and females; a reddish brown colored tail and a greenish iris; hemipenis reaching the 13th caudal plate.

Description of the holotype. BNHS 3304, an adult female, in a fairly good state of preservation. Head large (HL/SVL 0.05), longer than broad (HL/HW 1.41); triangular in shape and fairly distinct from a slender neck. Nostrils situated more towards the snout tip than the eyes (EN/ES 0.87). Eyes relatively small and oval (ED/HL 0.2), with a vertically elliptical pupil. Dorsal and lateral head scales smooth and imbricate. Rostral barely visible from above; followed by two large intranasals not in contact with each other. Fourteen cephalic scales in a line between the two elongated supraoculars. Nasal partially fused with the 1st supralabial; 12/12 supralabials; 3rd largest; 14/14 infralabials, of which, the first three contact the anterior chin shields. Loreal pit large and triangular. Two small postoculars; one preocular and crescent shaped subocular scales on either side of the head. Dorsal scales in 23:23:15 rows; with very feeble median longitudinal keels. Ventrals 173; broad and extending throughout the width of the belly; anal single; subcaudals 61; divided. Temporal scales small and smooth. Tail relatively short (TaL/TL 0.146) and prehensile.

Overall dorsal coloration dark grey in preservation, with a pale grey venter. Ventral surface of the tail lighter in color, bearing two incomplete white lateral stripes along the sides. Dorsal surface of the tail is a different color than the body, and of a lighter shade when compared to the body. Ventrolateral stripes absent on the body; white ventrolateral stripes present along the sides of the tail extending from the vent to the 13th subcaudal.

Variation. Measurements and scale counts of the paratypes and referred material are given in Table 1. Mid-body scale rows range from 21–25:21–23:15–17; ventrals range from 170–179 in males and 166–178 in females. Subcaudals range from 67–70 in males and 55–64 in females. Internasals usually in contact with each other, but separated by a small scale in one specimen. Cephalic scales range from 11–14; postoculars range from 2–3. Relative tail length in males: 0.178–0.200; in females: 0.143–0.161. Sexual dimorphism apparent in body for the tail dimensions and the number of subcaudals. Verdant green colored in life, both dorsally and ventrally, without a white ventrolateral pair of stripes along the sides of the body; but with a pair of white and red lateral stripes along the sides of the tail. Males have a thin white labial stripe bordered by red above the supralabials on either side of the head (absent in females)

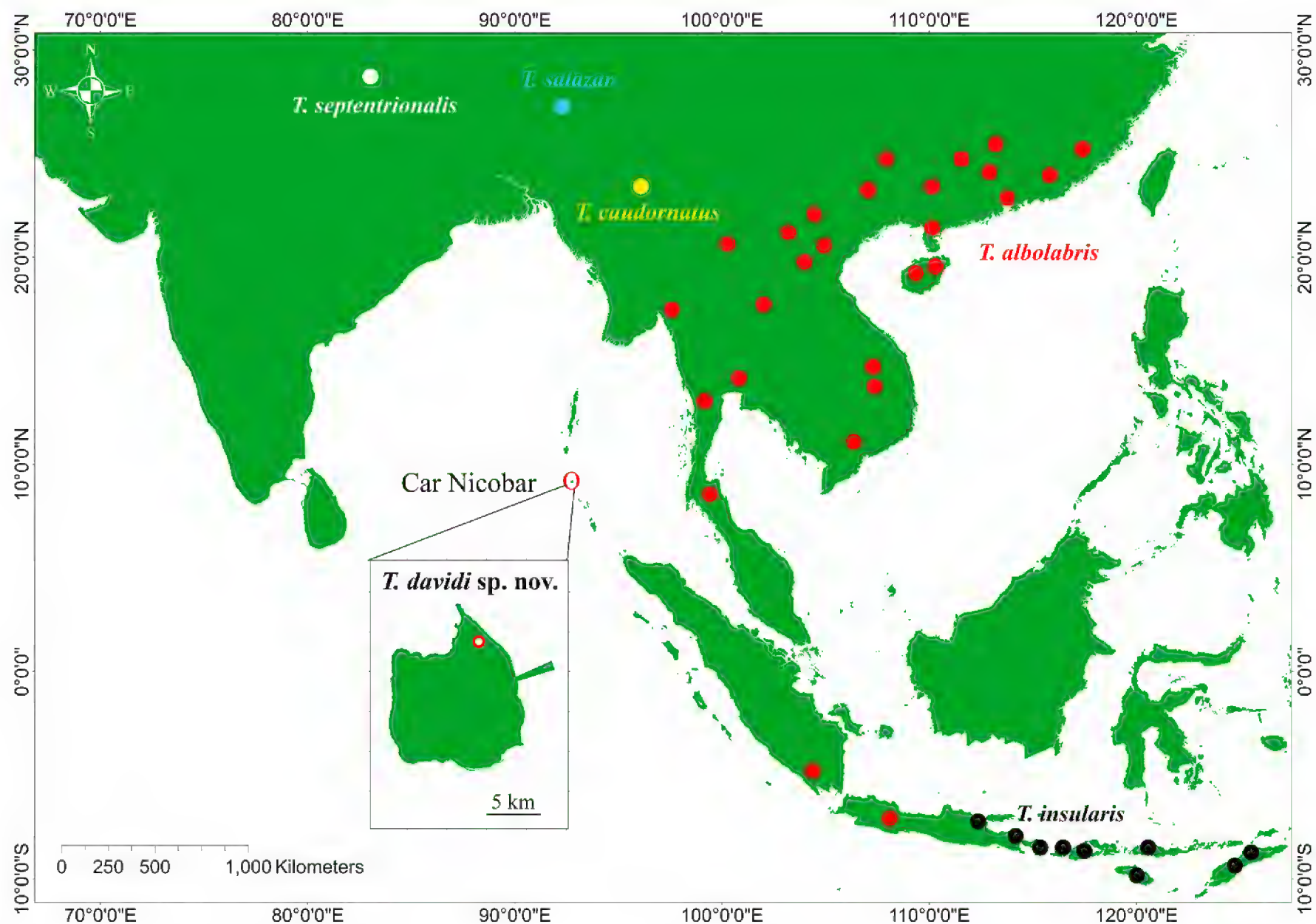


Fig. 3. Distribution of members of the *Trimeresurus albolabris* complex showing the type locality and distribution of *T. davidi* sp. nov.

and a white stripe bordered by red along the subcaudals from the vent until about half the length of the tail (also present in females).

Natural history and distribution (Fig. 3). Five individuals of *T. davidi* sp. nov. were encountered during this study. They were always observed as active and foraging during the night (2100 h and later) and were never encountered during the day. Individuals were seen on shrubs at heights ranging from 1.20 m ($n = 2$) to about 8 m ($n = 1$) above the ground; also seen on the rocky walls of old buildings ($n = 1$). One was found dead in a coconut plantation, presumably killed by someone. The large (~120 cm) female individual observed on the top of a tree at a height of about 8 m had a swollen anterior belly, indicating that it had fed recently. Lizards of the genera *Coryphophylax*, *Bronchocela*, *Cyrtodactylus*, and *Gehyra* were observed at close quarters (~2 m) from the point where the snakes were sighted. Other relatively small-bodied, endemic species of snakes, namely *T. labialis* (Fitzinger in: Steindachner, 1867) and *Lycodon tiwarii* Biswas and Sanyal, 1965, were observed to be sympatric with *T. davidi* sp. nov. and could be potential competitors as they are also nocturnal snakes feeding on prey species similar to *T. davidi* sp. nov.

Comparison. *Trimeresurus davidi* sp. nov. does not have any superficially similar looking, green-colored arboreal congeners on Car Nicobar Island, on which its

distribution is restricted. It can be distinguished from other members of the *T. albolabris* complex by the following combination of characters: dorsal scales of *T. davidi* sp. nov. in 21–25:21–23:15–17 rows (vs. 21–23:19–21:15 in *T. albolabris* and *T. insularis*, 21:21:15 in *T. caudornatus*, 21:19:17 in *T. septentrionalis*, and 21:19:15 in *T. salazar*). There is some overlap in this character, as is expected; however, five of the 11 (45%) examined *T. davidi* specimens had 23 dorsal scale rows at midbody. This character has never been recorded in any of the other species within this complex. Also, there seems to be a certain degree of overlap in scalation characters between the currently recognized members of *T. albolabris* complex, which makes the partially overlapping values with *T. davidi* sp. nov. quite understandable. *Trimeresurus davidi* sp. nov. has 166–179 ventrals (vs. 149–173 in *T. albolabris*, 161–163 in *T. caudornatus*, 160–181 in *T. septentrionalis*, 156–167 in *T. insularis*, and 163–171 in *T. salazar*); an absence of white ventrolateral stripes along the body in *T. davidi* sp. nov. (vs. present in *T. septentrionalis* and *T. salazar*); and the presence of a pair of red and white ventrolateral stripes along the sides of the tail (vs. absent in all other species). *Trimeresurus davidi* sp. nov. is considerably larger than all other species of this complex. For further comparisons, see also the morphological characters (Table 2) for the material examined (Appendix 1) in this study.

From the two other sympatric congeners, *T. andersoni*

Table 1. Measurements (in mm) and pholidosis of *Trimeresurus davidi* **sp. nov.** (* indicates holotype). Measurements for individuals with a regenerated/incomplete tail are shown in bold.

Voucher number	BNHS3304*	NHMUK 1936.7.7.40	NHMUK 1936.7.7.41	NHMUK 1936.7.7.42	Not collected	Chuckchucka	DOSMB 05104	Not collected	Chuckchucka	Not collected	NHMUK 1936.7.7.47	NHMUK 1936.7.7.48	NHMUK 1936.7.7.46
Locality	Chuckchucka	Car Nicobar	Car Nicobar	Car Nicobar	Chuckchucka	Chuckchucka	Chuckchucka	Chuckchucka	Chuckchucka	Chuckchucka	‘Nicobar Is.’	‘Andaman Is.’	‘Nicobar Is.’
Sex	F	F	F	F	F	F	M	M	M	M	M	M	unknown
SVL	593	750	680	835	360	770	334	277	421	459	459	459	372
Tail length	102	135	121	160	60	110	76	60	99	115	115	115	59
TaL/TL	0.146	0.153	0.151	0.161	0.143	0.125	0.185	0.178	0.190	0.200	0.200	0.200	0.137
Head length	27.37	41.22	36.5	47.21	17.82	43.68	15.4	14.0	21.64	23.58	23.58	23.58	20.44
Head width	19.36	29.15	26.28	31.7	12.36	26.34	13.16	10.32	13.38	12.82	12.82	12.82	10.87
Head depth	11.9	17.48	16	20.81	7.77	16.51	7.98	5.36	8.83	8.61	8.61	8.61	7.84
Eye diameter	5.25	4.48	3.9	5.15	3.33	4.54	2.84	3.19	3.25	3.75	3.75	3.75	2.83
Eye-nostril	7.19	8.17	7.2	9.68	3.37	8.77	3.56	3.2	4.1	5.11	5.11	5.11	3.36
Eye-snout	8.31	9.99	9.29	12.85	6.04	12.31	5.22	4.44	6.32	6.68	6.68	6.68	5.38
Interorbital distance	11.61	16.48	13.34	18.17	9.85	15.24	10	8.19	10.89	9.8	9.8	9.8	7.62
Internarial distance	4.23	11.19	11.85	8.89	3.59	7.03	3.8	3.26	3.33	4.25	4.25	4.25	2.75
Supralabials	12	11	10	12	10	10	10	10	10	11	11	11	10+
Infralabials	14	13	14	15	12	15	12	13	12	13	13	13	13
Preoculars	1	1	1	1	1	1	1	1	1	1	1	1	1
Postoculars	3	2	2	3	2	2	3	2	2	2	2	2	dry/damaged
Dorsal scale rows	23:23:15	23:21:15	25:23:16	25:23:17	21:21:17	23:23:15	25:23:15	21:21:15	24:21:15	21:21:15	21:21:15	21:21:15	22:22:16
Ventrals	173	173	170	166	168	178	179	175	172	170	170	170	168
Subcaudals	61	58	58	64	63	55	67	68	70	68	68	68	46
Anal	1	1	1	1	1	1	1	1	1	1	1	1	1
Subocular	1	1	1	1	1	1	1	1	1	1	1	1	1
Internasal	2	2	2	2	2	2	2	2	2	2	2	2	2
Internasals	separated	contact	contact	contact	contact	contact	contact	contact	contact	contact	contact	contact	contact
Cephalics	14	11	11	12	13	11	12	12	11	12	12	12	11

Table 2. Comparison of morphological characters within the *Trimeresurus albolabris* group, adapted from Chen et al. (2020), Mirza et al. (2020), and Grismer et al. (2008) in addition to the specimens examined during this study, the numbers of which are given under each species name.

Character	<i>T. davidi</i> sp. nov. <i>n</i> = 11	<i>T. albolabris</i> <i>n</i> = 46	<i>T. insularis</i> <i>n</i> = 7	<i>T. salazar</i> <i>n</i> = 6	<i>T. septentrionalis</i> <i>n</i> = 18	<i>T. caudornatus</i> <i>n</i> = 2
Mid-body scale rows	21–23	19–21	19–21	19–21	19–21	21
Ventrals	166–179	149–173	156–168	163–171	160–181	161–163
Subcaudals	58–70	48–67	54–75	59–74	55–83	52–72
SVL	277–835	297–668	418–613	363–415	454–675	425–537
TaL	60–160	31–146	115	60–94	104–197	77–122
TaL/TL	0.14–0.20	0.19–0.24	0.21–0.35	0.14–0.18	0.19–0.24	0.15–0.19
Ventrolateral body stripes	absent	present/absent	absent	present	present	absent
Ventrolateral tail stripes	present	absent	absent	absent	absent	absent

Theobald, 1868 and *T. labialis* Fitzinger in: Steindachner, 1867, *T. davidi* sp. nov. can be distinguished by its verdant green dorsal coloration (vs. predominantly brown in both *T. andersoni* and *T. labialis*); midbody dorsal scales in 21–23 rows (vs. 23–25 in *T. andersoni*, 23 in *T. labialis*); and the first supralabial united with the nasal in *T. davidi* sp. nov. (vs. separate in *T. labialis*).

Additionally, from the green color morph of *Trimeresurus cantori* (Blyth 1846) which occurs on islands of the central group of the Nicobar archipelago, *T. davidi* sp. nov. can be distinguished by a lower number of mid-body scale rows (21–23 in *T. davidi* sp. nov. vs. 25–29 in *T. cantori*); and the absence of a pair of white ventro-lateral stripes along the sides of the body in *T. davidi* sp. nov. (vs. present in *T. cantori*) [Whitaker and Captain 2008].

Discussion

Among the members of the genus *Trimeresurus*, *T. albolabris* has been and continues to be shown as comprising multiple cryptic lineages across its known distribution range in various parts of Southeast Asia (Kramer 1977; Zhu et al. 2016; Chen et al. 2020; Mirza et al. 2020). Currently, this complex comprises five species spanning a distribution across the Western Himalayan Nepal in the west to the Lesser Sunda Islands in the east. Herein, *T. davidi* sp. nov. has been added as a sixth member, occurring towards the southwestern extremity of the distribution range of the *T. albolabris* complex. This population has been known since Smith (1943: 524) mentioned six Nicobarese specimens, three males and three females, collected by Lord Moyne from Car Nicobar. He also remarked that these specimens lack the light flank stripe on the body, but have an unusually distinct one along the sides of the tail. The specimens described here from Car Nicobar also show this unique ventrolateral tail stripe.

Car Nicobar Island, to which *T. davidi* sp. nov. is restricted, is a small island of about 125 km². With a nearly flat terrain and a maximum elevation of about 90 m asl, it is fairly densely populated. However, Car Nicobar does

not have any protected areas such as national parks or wildlife sanctuaries and hence, a species such as *T. davidi* sp. nov. which is restricted only to this island is under a high level of threat. Unfortunately, the local Nicobarese people usually kill such snakes when encountered. Instances of bites from this species have been known (Vijayakumar and David 2006), and in one case a casualty was reported (Edmond, pers. comm.). According to the current data on its geographic distribution and abundance, we recommend that *T. davidi* sp. nov. be regarded as an Endangered species, following the criteria B1 (extent of occurrence < 5000 km²) and B2 (area of occupancy < 500 km²) of the IUCN guidelines, which indicates a need for immediate conservation attention. There are two other sympatric pit viper species on Car Nicobar, namely *T. labialis* Fitzinger in Steindachner, 1867 and *T. andersoni* Theobald, 1868 (Vogel et al. 2014). Of these, *T. labialis* shows a similar pattern of distribution to *T. davidi* sp. nov. by being endemic to this one single island, while the other species (*T. andersoni*) is known to occur throughout the Andaman archipelago as well (Whitaker and Captain 2008).

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Literature Cited

- Chen Z, Yu J, Vogel G, Shi S, Song Z, Tang Y, Yang J, Ding L, Chen C. 2020. A new pit viper of the genus *Trimeresurus* (Lacépède, 1804) (Squamata: Viperidae) from Southwest China. *Zootaxa* 4768(1): 112–128.
- David P, Vogel G, Dubois A. 2011. On the need to follow rigorously the Rules of the Code for the subsequent designation of a nucleospecies (type species) for a nominal genus which lacked one: the case of the nominal genus *Trimeresurus* Lacépède, 1804 (Reptilia: Squamata: Viperidae). *Zootaxa* 2992(1): 1–51.
- Dowling HG. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Grismer LL, Tri NV, Grismer JL. 2008. A new species of insular pitviper of the genus *Cryptelytrops* (Squamata: Viperidae) from southern Vietnam. *Zootaxa* 1715: 57–68.
- Kramer E. 1977. Zur Schlangenfauna Nepals. *Revue Suisse de Zoologie* 84(3): 721–761.
- Mirza ZA, Bhosale HS, Phansalkar PU, Sawant M, Gowande GG, Patel H. 2020. A new species of green pit vipers of the genus *Trimeresurus* Lacépède, 1804 (Reptilia, Serpentes, Viperidae) from western Arunachal Pradesh, India. *Zoosystematics and Evolution* 96(1): 123–138.
- Smith MA. 1943. *The Fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese subregion. Reptilia and Amphibia. Volume III. Serpentes*. Taylor and Francis, London, United Kingdom. xii + 583 p.
- Uetz P, Freed P, Hošek J. (Editors). 2020. The Reptile Database. Available: <http://www.reptile-database.org>. [Accessed: 10 February 2020].
- Vijayakumar SP, David P. 2006. Taxonomy, natural history, and distribution of the snakes of the Nicobar Islands (India), based on new materials and with an emphasis on endemic species. *Russian Journal of Herpetology* 13: 11–40.
- Vogel G. 2008. *Venomous Snakes of Asia / Giftschlangen Asiens*. Terralog Series 14. Edition Chimaira, Frankfurt am Main, Germany. 148 p.
- Vogel G, David P, Chandramouli SR. 2014. On the systematics of *Trimeresurus labialis* Fitzinger in Steindachner, 1867, a pitviper from the Nicobar Islands (India), with revalidation of *Trimeresurus mutabilis* Stoliczka, 1870 (Squamata, Viperidae, Crotalinae). *Zootaxa* 3786: 557–573.
- Whitaker R, Captain A. 2008. *Snakes of India – the Field Guide*. Draco Books, Chengalpeta, India. 481 p.
- Zhu F, Liu Q, Che J, Zhang L, Chen Z, Yan F, Murphy R, Guo C, Guo P. 2016. Molecular phylogeography of white-lipped tree viper (*Trimeresurus*; Viperidae). *Zoologica Scripta* 45: 252–262.



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Gernot Vogel was born in Heidelberg, Germany, obtained his Ph.D. in Chemistry, and is now working as a chemist. Beginning many years ago as a reptile keeper, he developed a great interest in the snake fauna of the Orient. His special interest lies in the systematics of snake genera which have large distribution areas, including *Trimeresurus*, *Boiga*, *Oligodon*, *Lycodon*, *Pareas*, *Dendrelaphis*, and others, with his main geographical emphasis on China, India, and Indonesia. He has published several papers specifically on the snakes of the Andamans.

Appendix 1. Comparative material examined.

Trimeresurus albolabris (17 specimens). China. NHMUK 1946.1.19.85, NHMUK 1946.1.23.73 (Syntypes) “China.” MNHG 1464.88–89 “Tung Kum, Canton.” NMW 23927, “Koksingas Port.” NMW 23905:2, 23905:5–7, “Hainan, Ting-An.” NMW 23626.4–5 “Hongkong.” ZMB 27669 “S-Kuang-tung.” ZMB 52600, “Fung Wan.” ZMB 66282 “Lu Kung, Katon.” ZMB 66283 “N-Kuantung.” Vietnam. CIB GV2019111704–5, “Tam Dao.”

Trimeresurus cf. *albolabris* (29 specimens). Vietnam. NMW 23901.8 “Phuc-Son, Annam.” NMW 23904.3–5, NMW 23920.7 “Annam.” NMW 23920.3 “Saigon.” Thailand. NMW 19528 “Thailand.” NMW 23901:3–4 “Dom Rek.” NMW 23926.1, NMW 23926.6–9, NMW 23930.1–2 “Pu-Kin.” NMW 27946.2–3, 27946.5–6 “Hills of Bangkok.” NMW 23898.1–2 “Don-Pia-Fei.” ZMB 70196 “Surat Thani.” Indonesia. NMW 23901.6, 23926.1–3 “Java.” MNW 23902 “Tasikmalaja, W Java.” RMNH 17189 “Sumatra.”

Trimeresurus septentrionalis (18 specimens). Nepal. CAS 135750 (Paratype) “Nähe Pokhara.” MHNG 1404.31. (Holotype) MHNG 1400.18, 24–26, 29–32, 34–39, 45, 47 (all Paratypes) “Nähe Pokhara.”

Trimeresurus insularis (7 specimens). Indonesia. NHMB 12773 (Holotype) “Soe, Timor.” NMW 39581 “Bali.” MNHN 4056, “Timor Island.” MNHN 4057, “Indes Orientales.” MNHN 2002.0402, “Wetar Island.” SMF 76352, 76353, “Flores Island.”

Trimeresurus erythrurus (22 specimens). India. NHMUK 1940.3.9.22 “Naga Hills.” NMHW 23903:1–2, Guwahati, Assam. ZSI 3052, ZSI 3002, ZSI 3013, ZSI 3045–46 “Samagooting, Assam.” ZMH R-6933 “Himalaya.” Myanmar. NHMUK 61.10.2.5–6, 1908.6.23.96 “Rangoon,” ZMH R-6934 “Rangoon.” CAS 220377, 240036, 204989 “Rakhin.” CAS 239352, 239502, 239511, 40120 “Ayeyarwaddi State.” CAS 240120 “Kakhim State.” CAS 243175 “Magway.”

Trimeresurus fasciatus (4 specimens). NHMUK 96.4.29.46 (Holotype), “Jampea Island,” now Tanahjampea, Province of Sulawesi Selatan, Indonesia. MNHN 1999.9071, MNHN 2002.0401–02, Tanahjampea, Province of Sulawesi Selatan, Indonesia, through the pet trade.



Forensic bioacoustics? The advertisement calls of two locally extinct frogs from Colombia

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Abstract.—Analyses of vocalizations are an important tool for anuran species taxonomy and identification, and can become especially important for detecting rare or threatened species. Based on recordings obtained in 1986 in the Reserva Natural La Planada (Department of Nariño, southern Colombia), we describe the acoustic characteristics of the advertisement calls of *Paruwrobates andinus* (Dendrobatidae) and *Gastrotheca guentheri* (Hemiphractidae), two Andean anuran species not seen since the 1990s and considered locally extinct. The call of *P. andinus* consists of a rapid series of short notes emitted in three call groups. The first two groups have five notes with 6–7 pulses each and a duration of about 2 sec, while the third call group contains 50 notes or more, with 5–8 pulses each and a duration of about 30 sec. On average, the notes have a fundamental frequency of 2.2 kHz and a dominant frequency of 4.4 kHz. This call differs from that of its likely most closely-related species, *P. erythromos*, in having shorter notes repeated at a higher rate. The call of *G. guentheri* has a single loud, short note (average duration, 0.262 sec) composed of 3–4 pulses, with negative frequency modulation, a fundamental frequency of 0.9 kHz, and a dominant frequency of 1.8 kHz. Advertisement calls of members of the *Gastrotheca longipes* group (to which *G. guentheri* belongs) are poorly known and seem to be quite variable, making it difficult to establish reliable comparisons.

Keywords. Amphibia, Andes, Dendrobatidae, extinction, *Gastrotheca guentheri*, Hemiphractidae, *Paruwrobates andinus*, vocalizations

Resumen.—Los análisis de las vocalizaciones son una importante herramienta en la taxonomía e identificación de anuros, y pueden ser especialmente importantes para detectar especies raras o amenazadas. A partir de grabaciones obtenidas en 1986 en la Reserva Natural La Planada (Departamento de Nariño, sur de Colombia), describimos las características de los cantos de *Paruwrobates andinus* (Dendrobatidae) y *Gastrotheca guentheri* (Hemiphractidae), dos especies de anuros andinos que no han sido observadas desde 1990 y se consideran extintas localmente. El canto de *P. andinus* consiste en series rápidas de notas cortas emitidas en tres grupos de llamadas, las dos primeras conteniendo 5 notas con 6–7 pulsos cada una, y una duración de cerca de 2 segundos, mientras que el tercer grupo contiene 50 notas o más, con 5–8 pulsos cada una y una duración de cerca de 30 segundos; en promedio, la frecuencia fundamental es de 2.2 kHz y la frecuencia dominante es de 4.4 kHz. Este canto difiere del de la que probablemente es la especie más próxima, *P. erythromos*, por tener notas más cortas repetidas a un ritmo más rápido. El canto de *G. guentheri* tiene una sola nota alta y corta (duración media, 0.262 segundos) compuesta por 3–4 pulsos, con modulación de frecuencia negativa, frecuencia fundamental de 0.9 kHz y frecuencia dominante de 1.8 kHz. Los cantos dentro del grupo de especies de *Gastrotheca longipes* (al cual pertenece *G. guentheri*) no se conocen bien y parecen ser muy variables, por lo que no se pueden hacer comparaciones fiables.

Palabras clave. Amphibia, Andes, Dendrobatidae, extinción, *Gastrotheca guentheri*, Hemiphractidae, *Paruwrobates andinus*, vocalizaciones

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Introduction

“Extinction is forever” is a famous sentence often quoted in texts on evolution and conservation biology, but deciding when a species is extinct is not an easy task. Empirical data, either on the existence of surviving individuals or their disappearance, are often difficult to obtain, especially for poorly known, rare, widespread, or elusive species. While the conservation status of species with small or restricted distributions can be more easily evaluated, surveying the entire range of species with large and/or patchy distributions in order to know whether they are extant or extinct is a much greater challenge. For this reason, a more conservative and less compromising approach is simply to refer to local extinctions or extirpations, instead of extinction (see Smith-Paten et al. 2015).

Within the current Sixth Mass Extinction crisis, amphibians are the most threatened class of vertebrates due to several factors (Wake and Vredenburg 2008). In particular, chytridiomycosis has been linked to the extinction of at least 90 species of amphibians and the decline of 501 others (Scheele et al. 2019). The amphibian crisis is especially severe in the American tropics, where mountain forest ecosystems, even pristine ones, have suffered the collapse of entire amphibian communities (e.g., Lips 1998, 1999; Lips et al., 2006, 2008; Catenazzi et al. 2011, 2014), usually including precipitous declines of many species and the extirpation of others. Because the beta-diversity is high and many amphibian species have reduced distributions in tropical mountains, in many cases local extirpation equals the extinction of the species altogether.

One case of amphibian community collapse in the tropical Andes which remains poorly documented is that of the Reserva Natural La Planada, in southern Colombia (Fig. 1A–B). In 1986, during a three-month period from April to June, Patricia Burrowes carried out the first (and hitherto only) comprehensive study of the amphibian community of La Planada (Burrowes 1987). A total of 42 species of amphibians were reported, including 12 species of anurans new to science (Myers and Burrowes 1987; Duellman and Burrowes 1989; Lynch and Burrowes 1990). After 1986, further amphibian monitoring and inventory in La Planada has been anecdotal and intermittent, mostly carried out by G. Cantillo and field parties from the Universidad de Nariño (e.g., Muñoz-Arcos et al. 2016). In April 2019, four of the authors of this article (IDIR, PAB, GC, and BC) and several students returned to La Planada to carry out amphibian surveys. Although the temporal sampling effort was much shorter than in 1986, that brevity was partially compensated by having a large team instead of a single person doing the fieldwork. Preliminary data indicated that massive declines in numbers and species diversity have taken place in La Planada, suggesting chytridiomycosis and climate change as potential factors

involved in the observed collapse (De la Riva and Burrowes 2019; data not shown).

Two of the most iconic anurans studied in La Planada by Burrowes in 1986 were the poison arrow frog, *Paruwrobates andinus* (Myers and Burrowes, 1987) [Fig. 1C], and the marsupial frog, *Gastrotheca guentheri* (Boulenger, 1882) [Fig. 1D]. *Paruwrobates andinus* was described (as *Epipedobates andinus*) by Myers and Burrowes (1987) based on nine specimens. This small species (snout-to-vent length [SVL] of males 19.5–20.1 mm; females, 20.7–21.5 mm) is considered diurnal, terrestrial, and semiarboreal, being observed near water-filled bromeliads in trees and near fallen tree branches (Myers and Burrowes 1987). The species is only known from La Planada, where it was observed across an elevation range of 1,700–2,020 m asl (Myers and Burrowes 1987; Lötters et al. 2007; Kahn et al. 2016; Frost 2020). It has not been seen at the type locality since the 1990s (G. Cantillo, field notes), and its conservation status is “Critically Endangered (Possibly Extinct)” according to IUCN (2019a). While it is possible that the species still occurs somewhere in southwestern Colombia or even nearby northwestern Ecuador, it has not been found despite several search attempts, both in La Planada since 2013 and at nearby Río Nambí Natural Reserve (IUCN 2019a). Thus, this is an example of a species extirpated locally, and perhaps extinct.

The case of *Gastrotheca guentheri* is quite different. The species has been known since its original description as the only anuran with true teeth in the lower jaw, which led Boulenger (1882) to place it in its own genus, *Amphignathodon*, meaning “teeth in both jaws,” a phenomenon studied and discussed by Wiens (2011). Despite this exceptional peculiarity, different phylogenetic analyses have consistently placed the species deeply nested in the genus *Gastrotheca* (e.g., Duellman et al. 1988; Wiens et al. 2007; Castroviejo-Fisher et al. 2015). This moderately large species (SVL of males 67.8–76 mm; females, 69.9–82 mm) is nocturnal and usually associated with canopy vegetation, including bromeliads, and frequently found next to rivers (Arteaga et al. 2013; Duellman 2015). It preys on small vertebrates, such as frogs and lizards, and large insects, such as orthopterans (Arteaga et al. 2013; Paluh et al. 2019), a feeding behavior expected by Wiens (2011) based on the functional teeth in the lower jaw which enable the species to catch and swallow large prey. *Gastrotheca guentheri* occurs between 1,200–2,010 m asl in Andean cloud forests along the Cordillera Occidental from provinces Cotopaxi, Imbabura, and Pichincha in northwestern Ecuador to the Department of Antioquia in northwestern Colombia. A distribution gap of about 500 km has been noted between a Nariño-Cauca nucleus in the south of Colombia and a nucleus in the north comprising the departments of Risaralda-Chocó-Antioquia. However, based on specimen IND 4853, collected by J.V. Rueda on 3 December 1989 and examined by two of the authors

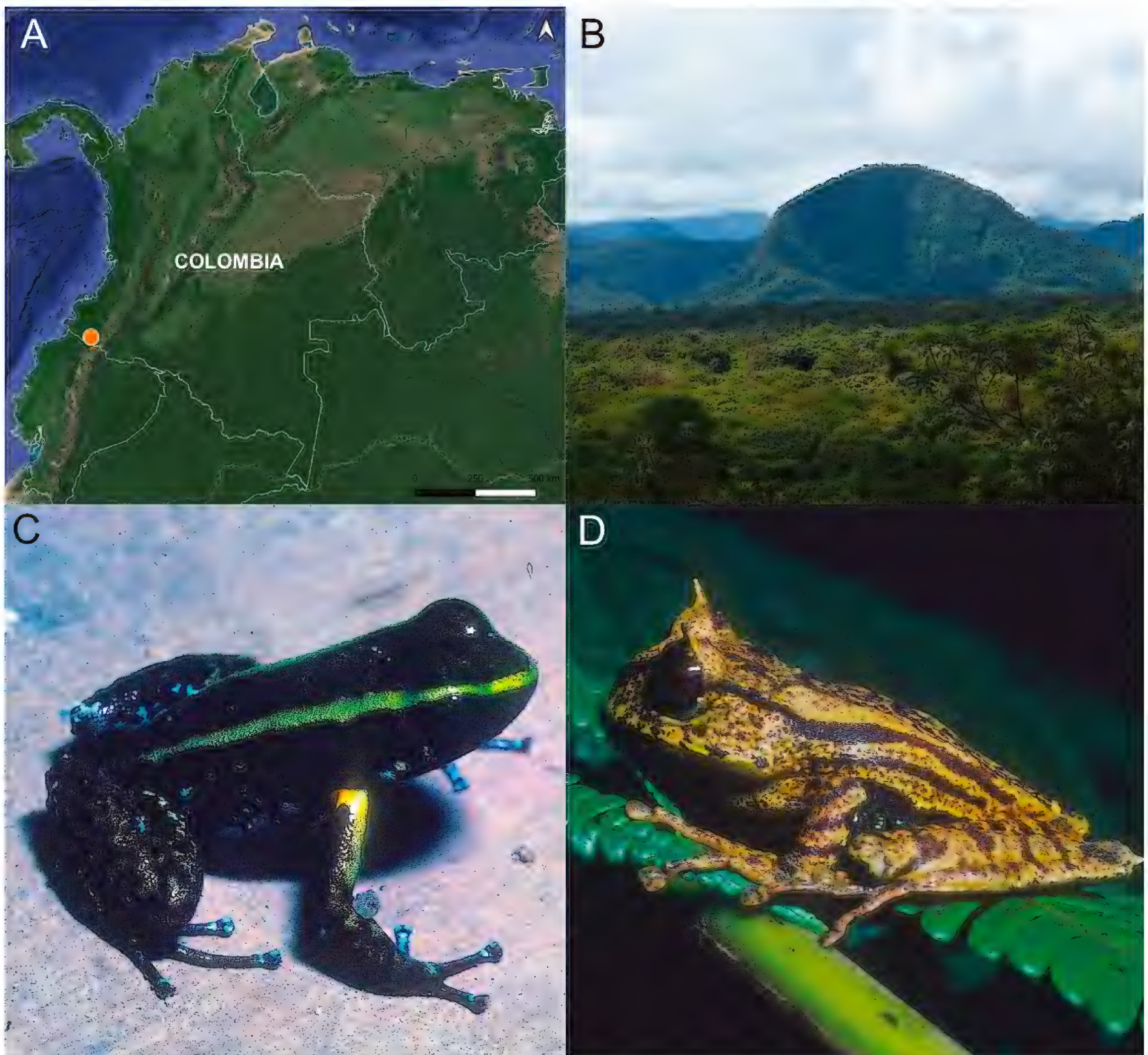


Fig. 1. Geographic location (A) and general view (B) of Reserva Natural La Planada (Department of Nariño, Colombia; (C) *Paruwrobates andinus* and (D) *Gastrotheca guentheri* from Reserva Natural La Planada, Colombia. Photos by I. De la Riva (B) and P.A. Burrowes (C–D).

(PAB and IDIR) at the herpetological collection of the Institute Von Humboldt, the species is also recorded in Risaralda (see below). A dubious record exists from the Amazonian slopes of northeastern Ecuador (Duellman 2015; IUCN 2019b; Frost 2020).

Gastrotheca guentheri is globally considered as Data Deficient, although its situation differs in Ecuador and Colombia (IUCN 2019b). In Ecuador, the species is considered as possibly extinct, because it has not been observed since 1996 despite active searches at known localities (Arteaga et al. 2013; IUCN 2019b), and the most recent specimen was collected on 1 January 1991 (L. Coloma, pers. comm.). In Colombia, its conservation status is difficult to assess due to its broader and apparently patchy distribution. It was last seen in 1990, and was not found in surveys carried out in La Planada in 2005 or in Parque Nacional Natural Munchique (Department of

Cauca) in 2014–2016 (IUCN 2019b). Two specimens deposited at the Instituto de Ciencias Naturales-Universidad Nacional de Colombia [ICN 50102–3] were collected in Munchique in 1990 (A. Acosta, pers. comm.), although the species was not reported in the park by Pisso et al. (2018). The disappearances of the species at some locations may be explained by pollution and habitat loss, but those at pristine habitats are attributed to climate change or/and chytridiomycosis (IUCN 2019b).

In 1986, P. Burrowes and G. Cantillo obtained recordings in La Planada of *P. andinus* and *G. guentheri*, two species for which the advertisement calls were then unknown. Later, the general vocal behavior of *P. andinus* was outlined by Myers and Burrowes (1987), who described the vocalizations of the species as “a distinctive call comprised of a series of well-spaced ‘creek’ notes” often emitted from bromeliads at different heights in

the trees. As for *G. guentheri*, the call was described by Duellman (2015) as “a single loud ‘bop,’ usually repeated at intervals of several minutes,” although “some individuals have been heard to produce two or three calls in quick succession.”

This paper provides the first comprehensive analyses, including the first numerical descriptions, of the advertisement calls of these two species, which are now apparently extirpated from La Planada, and might be extinct altogether. Because observing frogs is usually harder than hearing them, this case study of what can be termed “forensic bioacoustics” may prove useful in future surveys and acoustic monitoring of anurans in the Pacific Andean forests of Colombia and Ecuador.

Materials and Methods

The study site, Reserva Natural La Planada, is a protected area of 3,200 hectares at 1,700–2,010 m asl, in the cloud forests of the Pacific slopes of the Andes, Department of Nariño, Colombia (1°09'29"N, 77°58'36"W), near the Ecuadorian border (Fig. 1A).

The recordings were obtained from individuals (adult males in both cases) kept in captivity on 7 May 1986 at 1915 h for *G. guentheri*, and on 5 June 1986 at 1030 h for *P. andinus*. Unfortunately, records were not made of the air temperature or the size of the recorded specimens. The sound files were originally obtained by means of a Sony stereo cassette recorder TCS-350 and stored in analog cassettes, then digitized in a lossy compressed format (mp3). Because the cassettes were not available at the time of our study, the *.mp3 files were finally converted to *.wav for analyses with Raven 1.05 software (Cornell Lab of Ornithology 2014). Figures were generated with the R package Seewave (Suerur et al. 2008). Temporal data were obtained from the oscillograms and frequency information was obtained using fast Fourier transforms (FFTs; frame width: 1,024 points).

The terminology used for the call descriptions is based on Köhler et al. (2017) and the oscillograms and audiospectrograms presented follow the format of Bosch et al. (2000). Due to the compression imposed by the MP3 format, some of the properties of the recordings could be affected as reported by Araya-Salas et al. (2019). Several parameters were considered for the analyses of the calls: call group duration, intercall group duration, note repetition rate, internote duration, dominant frequency, and fundamental frequency. To determine the point in which the amplitude is maximum within each call/note three points were used: at the beginning of the call (t1), at the middle section (t2), and at the end (t3); thus, providing some information about frequency modulation (t3 minus t1). In addition, the delta harmonic energy (dB) was measured as the difference between the peak intensity of the dominant frequency minus the peak intensity of the fundamental frequency. This parameter provides information about the energy distribution in

the spectrum. The number of pulses within calls/notes and pulse rate (pulses per sec) within calls/notes were difficult to estimate because the pulses were not clearly delimited due to their incomplete amplitude modulation. The numerical parameters of the advertisement calls are shown in Tables 1 and 2. The acoustic characteristics of the advertisement calls of *P. andinus* and *G. guentheri* were compared with those of other species which are phylogenetically closely related to them.

The original recordings are deposited in the scientific collection of the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales-CSIC, with collection numbers 11998 (*P. andinus* call) and 11997 (*G. guentheri* call) and are available in the web checklist *Frog Calls of the World* at the following links for *P. andinus* (http://www.fonozoo.com/fnz_detalle_registro_eng.php?tipo_registro=2&id=22942&id_sonido=1116) and *G. guentheri* (http://www.fonozoo.com/fnz_detalle_registro_eng.php?tipo_registro=2&id=22943&id_sonido=1117).

Results

The description of the call of *P. andinus* is based on the analysis of two call sequences. Only one type of call was heard, and three call groups were identified: A and B are similar to each other, while C is markedly different regarding the number of notes. Call groups A and B are composed of five notes with 6–7 pulses each, while call group C is longer, encompassing 53 notes with 5–8 pulses each (Fig. 2). For the analyses, only 50 notes were considered due to the background noise. The sounds of these individual notes are very similar in all call groups, having an average duration of 0.118 sec per note and an average inter-note duration of 0.450 sec ($n = 73$). The envelope shape of the audiospectrogram is slightly asymmetrical, with rise time faster than fall time. An average call group duration of 2.089 sec ($n = 2$) was registered for call groups A and B, and a call duration of 30.139 sec for call group C. The inter-call group duration between A and B is 2.247 sec ($n = 2$), and 1.778 sec between B and C. Inter-call group durations were considered from the end of one call group to the beginning of the next call group. The note repetition rate in group C is 1.76 notes/sec. Inter-note durations were measured from the end of one note to the beginning of the next one. All of the call groups contain a series of notes with an average fundamental frequency around 2.2 kHz, a dominant frequency of 4.3 kHz, and one harmonic around 6.5 kHz. The presence and relative power of other harmonics were difficult to ascertain because of the MP3 format compression and the likely automatic gain control used in the recorder. The average delta harmonic energy is 29.9 dB (SD \pm 8.246, range 17.1–46.4) and the average frequency modulation is 222.2 Hz (SD \pm 129.6, range 129.2–344.5). In call group C, there is an increase in the amplitude level possibly due to a closer approach of the

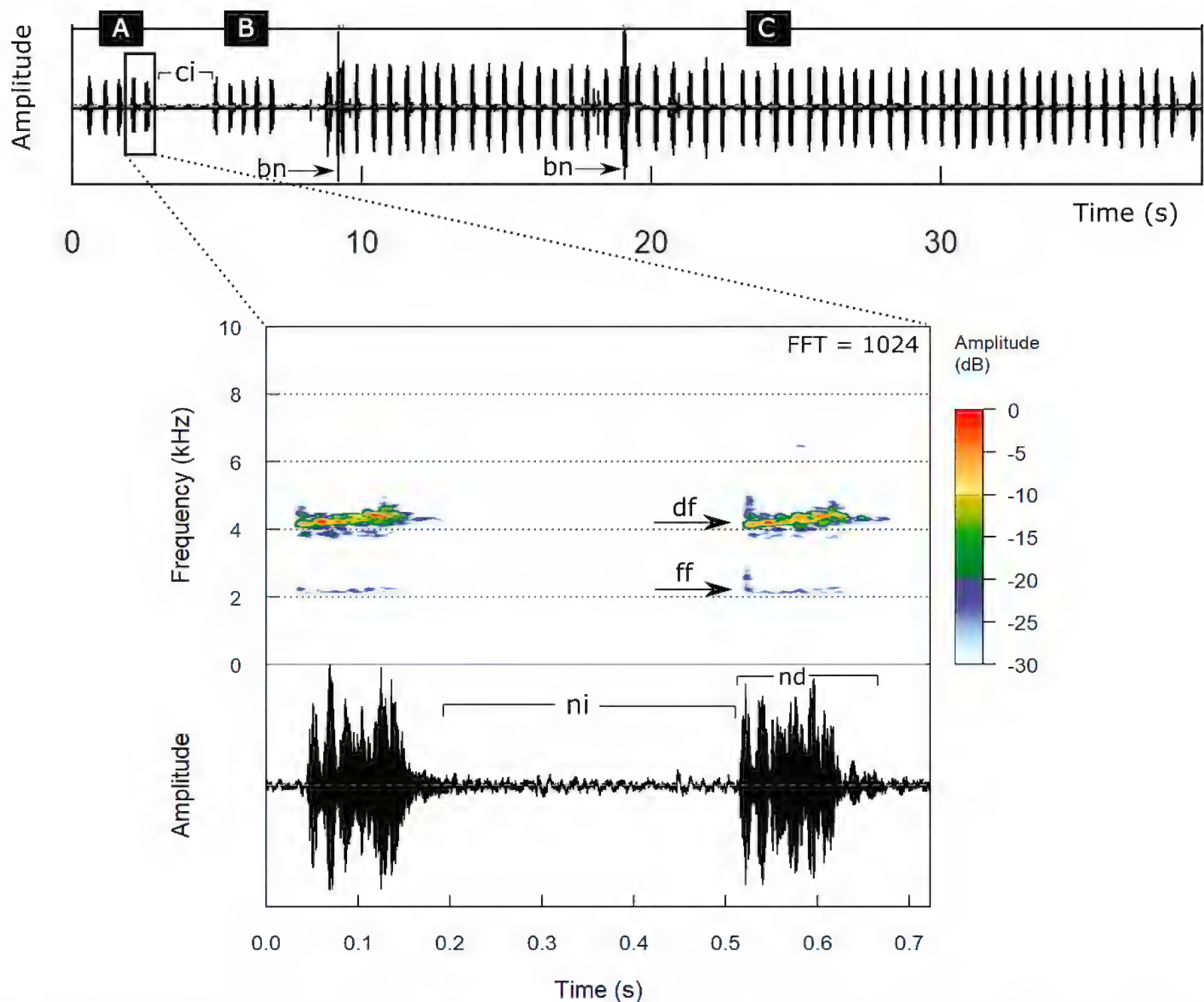


Fig. 2. Full-scale oscillogram (top), and expanded oscillogram and its audiospectrogram (bottom) of the advertisement call of *Paruwrobates andinus*. Call groups (A, B, and C), inter-call group interval (ci), and background noise (bn) are represented in the full-scale oscillogram. The note duration (nd), inter note interval (ni), dominant frequency (df), and fundamental frequency (ff) are indicated in the expanded box.

recorder to the sound source. The numerical data of the advertisement calls are summarized in Table 1.

At La Planada, the loud call of *G. guentheri* could be heard coming from the canopy and, despite it being a mostly nocturnal species, some individuals started to vocalize as early as 1400 h when raining (data not shown). The analyses here are based on two recorded calls. Only one type of call was heard, and each call was composed by only one note constituted by 3–4 pulses (Fig. 3). The envelope shape of the audiospectrogram is asymmetrical, with a fast rise time and a more extended fall time. Notes have an average duration of 0.262 sec, an average fundamental frequency around 0.9 kHz, and a dominant frequency of 1.8 kHz. The presence of other harmonics was difficult to measure due to the same issue as in the *P. andinus* recordings. Frequency modulation is negative, and amplitude modulation is also predominately negative (see the descendant envelope shape in the oscillogram, Fig. 3). The inter-call duration, considered between the end of the first call and the beginning of the second one, was 20.4 sec. However, due to the fact that only a

sequence of two calls was recorded, we cannot consider that this inter-call duration is representative. The average delta harmonic energy is 6.4 dB and the average frequency modulation is -0.2 kHz. The numerical data of the advertisement calls are summarized in Table 2.

Discussion

Recordings and knowledge of anuran advertisement calls not only constitute a powerful taxonomic tool, but call parameters can also contain valuable phylogenetic information. However, anuran advertisement calls are subjected to strong sexual selection, which can promote rapid character differentiation even in species which are closely related, especially if they occur in sympatry (see Goicoechea et al. 2010, and references therein). On the other hand, similar selective pressures affecting the evolution of advertisement calls of non-related species can lead to high levels of homoplasy in particular bioacoustic parameters. Thus, when comparing anuran vocalizations, determining which similarities are due to

Calls of two extirpated Colombian frogs

Table 1. Summary of numerical parameters of vocalizations of *Paruwrobates andinus* and some other species of dendrobatoid frogs (mean \pm SD, range). Acoustic data of *P. erythromos* were extracted from Myers and Burrowes (1987); of *E. atopoglossus* from Grant et al. (1997); of *E. isthminus* from Myers et al. (2012); and of the species within the *Ameerega picta* group from Serrano-Rojas et al. (2017). *Note: In *P. andinus*, the inter-call interval represents the value of the mean inter-call group interval of this species.

	Note duration (s)	Inter-note interval (s)	Note repetition rate (notes/s)	Inter-call interval (s)	Dominant frequency (Hz)	Fundamental frequency (Hz)
<i>Paruwrobates andinus</i>						
Mean \pm SD	0.118 \pm 0.011	0.45 \pm 0.113	1.76	2.013 \pm 0.331	4,323.8 \pm 774.0	2,169.1 \pm 405.0
Range	0.105–0.157	0.31–0.58	-	1.778–2.247*	4,134.4–4,478.9	2,067.2–2,756.2
	<i>n</i> = 60	<i>n</i> = 60	-	<i>n</i> = 2	-	-
<i>Paruwrobates erythromos</i>						
Mean \pm SD	0.135 \pm 0.007	1.3 \pm 0.141	0.63	-	4,166.7 \pm 611.0	-
Range	0.13–0.14	1.2–1.4	-	-	3,500–4,700	
<i>Ectopoglossus atopoglossus</i>						
Mean \pm SD	\leq 0.03	-	14.8	-	4,733.3 \pm 924.6	-
Range	-	-	-	-	4,160–5,800	
<i>Ectopoglossus isthminus</i>						
Mean \pm SD	0.085 \pm 0.007	-	1.3	-	4,166.7 \pm 642.9	-
Range	0.08–0.09	-	-	-	3,700–4,900	
<i>Ameerega shihuemoy</i>						
Mean \pm SD	0.098 \pm 0.007	1.04 \pm 0.19	0.9 \pm 0.1	1.04 \pm 0.187	4,672.7 \pm 251	4,237 \pm 281.9
Range	0.084–0.12	0.97–1.2	0.8–1.0	-	4,478.9–4,909.6	-
<i>Ameerega simulans</i>						
Mean \pm SD	0.105 \pm 0.01	-	1.3 \pm 0.1	0.691 \pm 0.163	4,460.3 \pm 157.7	4,060.9 \pm 74.6
<i>Ameerega picta</i>						
Mean \pm SD	0.046 \pm 0.002	-	2.2 \pm 0.1	0.430 \pm 0.045	4,044.2 \pm 94.7	3,770.7 \pm 76.7
<i>Ameerega hahneli</i>						
Mean \pm SD	0.013 \pm 0.003	-	8.5 \pm 0.1	0.107 \pm 0.013	4,550 \pm 49.1	2,516.8 \pm 83.7
<i>Ameerega boliviana</i>						
Mean \pm SD	0.081 \pm 0.009	-	1.2 \pm 0.1	0.783 \pm 0.089	3,846 \pm 46.3	3,416.1 \pm 68.2
<i>Ameerega yungicola</i>						
Mean \pm SD	0.048 \pm 0.005	-	5.2 \pm 0.0	0.148 \pm 0.007	3,703.7 \pm 0.0	3,475.7 \pm 43.5
<i>Ameerega macero</i>						
Mean \pm SD	0.038 \pm 0.001	-	8.7 \pm 0.0	0.076 \pm 0.003	3,617.6 \pm 0.0	3,353.7 \pm 38.1

convergence and which ones represent a phylogenetic signal can be contentious. In any case, it is useful to make call comparisons of species that are closely related and, if they have allopatric distributions, the observed similarities may support hypotheses of relatedness based on other sources of evidence. The two species studied herein are rare and their closest relatives are also poorly known; as a consequence, they have not been thoroughly studied from a bioacoustics standpoint. However, some inferences can be made from comparing the data available at hand.

Parubrowates andinus was originally described by Myers and Burrowes (1987) in the genus *Epipedobates*, which was then a diverse and broadly distributed group in South America; however, it was later split into various genera, one of them being *Ameerega*, in which the species was included as *Ameerega andina* (Frost et al. 2006; Grant et al. 2006). The genus *Ameerega* was

subsequently rearranged by Grant et al. (2017), and *Paruwrobates* (a genus described by Bauer [1994] to include the species from La Planada) was resurrected from its synonymy to accommodate only three trans-Andean species: the Colombian *P. andinus* (type species), and the Ecuadorian *P. erythromos*, and *P. whymperi*. Edwards (1971) included *P. whymperi* in the genus *Colosthetus*, and Coloma (1995), in his review of Ecuadorian species of this genus, had already pointed out the similarity of this species with *P. erythromos* (then in the genus *Epipedobates*). Unfortunately, the call of *P. whymperi* is unknown. Finally, Grant et al. (2017) suggested that the colorful, aposematic Colombian species *Colosthetus ucumari* might belong to *Paruwrobates*, but its call is also unknown.

At the moment, phylogenetic hypotheses of dendrobatoid frogs are mostly based on morphological and genetic characters, with the occasional support of

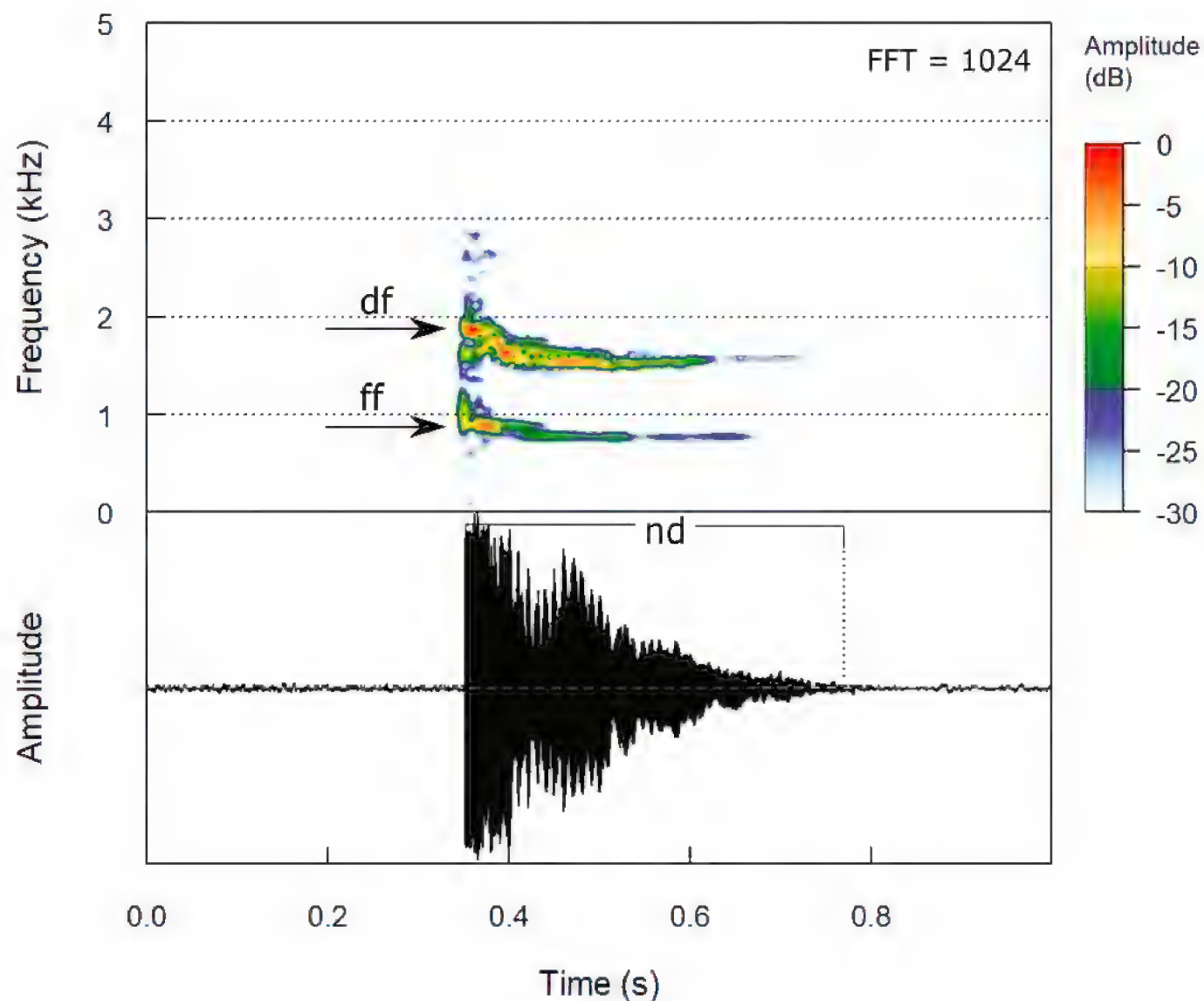


Fig. 3. Full-scale audiospectrogram (top) and oscillogram (bottom) of the advertisement call of *Gastrotheca guentheri*. The note duration (nd), dominant frequency (df), and fundamental frequency (ff) are indicated.

data from skin alkaloids (see Grant et al. 2006, 2017, and references therein). Myers and Burrowes (1987) described the call of *P. erythromos* (as *Dendrobates erythromos*) and opportunely considered it useful to compare this call with that of the new species from La Planada, whenever recordings were available. In fact, the recordings analyzed here had been obtained by the time of the description of *P. andinus*, but they were then “in storage” and only now available to be formally described. They now provide an opportunity to make the desired comparisons in order to ascertain whether the allocation of *P. andinus* and *P. erythromos* in the same genus is somehow supported by bioacoustical data as an additional source of evidence. This study also compares the call of *P. andinus* with those of two (out of seven) representatives of *Ectopoglossus* (*E. atopoglossus* and *E. isthminus*), another trans-Andean and Panamanian genus which is the sister group of *Paruwrobates* (Grant et al. 2017; Frost 2020). At the end of the convoluted taxonomic trajectory of many groups of dendrobatid frogs, *Ameerega* and *Paruwrobates* have ended up in different subfamilies, Colosthetinae and Hyloxalinae, respectively, within the family Dendrobatidae (Grant et al. 2017; Guillory et al. 2019; Frost 2020). Because of this distant phylogenetic

relatedness, the calls of *P. andinus* are compared with those of some representatives of *Ameerega* for descriptive purposes only; searching for possible shared character states, either as a result of having a common ancestor or of convergence, is beyond the scope of this paper.

Comparing the calls of *P. erythromos* and *P. andinus*.

The call of *P. erythromos* was verbally described as a series of well-spaced “short repetitive chips” (Vigle and Miyata 1980), and then a 3.6 min sequence was analyzed and figured by Myers and Burrowes (1987). These authors described it as follows:

“The advertisement call is a long train of harsh but not very loud ‘chirps,’ given continuously for many seconds. The one recording made includes an unbroken sequence of 136 notes given in 3 min, 35 s, for an overall repetition rate of 0.63 notes/s. Spacing between notes varied from 1 to 4 s, with an internote interval of about 1.2–1.4 s being most typical. Individual notes are 0.13–0.14 s duration and have a median frequency of about 4.5 kHz. Frequency is modulated within the note, there being a slow rise and more rapid fall, as indicated by the frequency-time

Table 2. Mean ± standard deviation (SD) and range of call parameters of *Gastrotheca guentheri* (n = 2).

Note duration (s)	Intercall (s)	Dominant frequency (Hz)	Fundamental frequency (Hz)	Delta harmonic energy (dB)	Frequency modulation (Hz)
0.262 ± 0.015	20.411	1,765.7 ± 182.716	861.3 ± 0.00	6.4 ± 2.121	-215.3 ± 182.716
0.273–0.251		1,636.5–1,894.9	861.3–861.3	4.9–7.9	(-344.5)–(-86.1)

curvature on spectrograms [...]; sections of a few notes show the dominant frequency starting at about 4,300 Hz, rising to about 4,700 Hz, then dropping back below 4,500 Hz. The individual note is strongly pulsed at a rate of 131 pulses/s; the first several pulses are produced more rapidly than subsequent ones.”

Comparisons of anuran calls among different studies would be easier if the same terminology and forms of graphical representation were used, but we are still far from reaching this desirable standardization (see Köhler et al. 2017). The terminology and type of figure that Myers and Burrowes (1987) used to describe the call of *P. erythromos* differ from those presented herein. However, a careful reading of their description of the call, and a visual comparison of their Fig. 12 (Myers and Burrowes [1987, p. 15]) with the audiospectrogram in Fig. 2 of this paper, allows for useful comparisons. Both calls consist of a rapid succession of short notes. In the temporal domain, the call of *P. andinus* has slightly shorter notes (0.118 sec on average, vs. 0.13–0.14 sec in *P. erythromos*), they are composed by fewer pulses (5–8 vs. 17, in the figure by Myers and Burrowes cited above), the inter-note intervals are shorter (0.45 sec on average vs. 1–4 sec, but most often 1.2–1.4 sec), and notes are repeated at a higher rate (note repetition rate 1.76 notes/sec vs. 0.63 notes/sec). Data are not available on the temperatures at the times of recording in either species. However, despite the known general effect of temperature on the behavioral and physiologically-induced variation of gross temporal parameters of anuran calls (the higher the temperature, the faster the call [Gerhardt 1994]), the differences found here are sufficiently remarkable to affirm that the call of *P. andinus* is notably faster than that of *P. erythromos*. In the spectral component, both species have a similar dominant frequency around 4.3 kHz, but *P. andinus* shows a narrower frequency modulation (0.2 kHz vs. 0.4 kHz in *P. erythromos*), and the envelope of the audiospectrogram shows a more complex call in *P. erythromos*, with a slow rise at the beginning of the note and a more rapid fall at the end.

Comparison with calls of *Ectopoglossus* species. Advertisement calls have been described for two species of the genus *Ectopoglossus*, the sister group of the genus *Paruwrobates* (Grant et al. 2017). The calls of those two species are quite different from each other, and also differ noticeably from those of *P. andinus* and *P. erythromos*. The call of *E. atopoglossus* was described by Grant et al. (1997). It consists of a fast succession of 12–14 short notes (note duration < 0.03 sec) repeated at a high rate (note repetition rate 14.8 notes/sec, as deduced from call duration and notes per call), emitted with a rising frequency from 4.16–4.24 kHz in the first notes of the call to 5.80 kHz in subsequent notes and with a decline in the last notes. The call of *E. isthminus* was described by Myers et al. (2012), and consists of a long

train of weakly pulsed (3 pulses/note) short notes (note duration 0.08–0.09 sec) repeated at a much lower rate than in *E. atopoglossus* (1.3 notes/sec); frequency is also modulated, rising sharply from 3.7–3.9 kHz to 4.9 kHz.

In summary, the calls of species of *Paruwrobates* and *Ectopoglossus* share the common feature of being composed of long trains of pulsed notes; in *Paruwrobates* notes are moderately long (one order of magnitude longer than those of *Ectopoglossus*) and frequency modulation is not very marked. In contrast, species of *Ectopoglossus* share calls composed of short notes with remarkable frequency modulation but highly variable note repetition rates. Whether these similarities and differences can be explained by phylogenetic relatedness is difficult to ascertain.

Comparison with calls of *Ameerega* species. Species of *Paruwrobates* were formerly placed in the genus *Ameerega* (Grant et al. 2017; Guillory et al. 2019). Serrano-Rojas et al. (2017) provided call analyses of several species of *Ameerega* in the *A. picta* group (a group not recognized by Guillory et al. 2019). In comparison to those species of *Ameerega* analyzed by Serrano-Rojas et al. (2017), the advertisement call of *P. andinus* has, in general, a longer note duration and inter-call duration, sustains the highest fundamental frequency, and has a dominant frequency which falls within the dominant frequency range (Table 1). However, considering the great heterogeneity of call characteristics shown even by closely related species of *Ameerega*, comparing their calls with those of *P. andinus* does not shed much light on their phylogenetic relationships.

Comparisons of calls of *G. guentheri* with those of other *Gastrotheca* species. *Gastrotheca guentheri* was included by Castroviejo-Fisher et al. (2015) in the *Gastrotheca longipes* group (equivalent to the subgenus *Amphignathodon* used by Duellman [2015]), which also comprises *G. andaquiensis*, *G. angustifrons*, *G. antomia*, *G. bufona*, *G. cornuta*, *G. dendronastes*, *G. helenae*, *G. longipes*, *G. walkeri*, *G. weinlandii*, and *G. williamsoni*. According to Castroviejo-Fisher et al. (2015), the species most closely related to *G. guentheri* is *G. weinlandii*. However, as far as we know, the only one of these species for which bioacoustic characteristics have been comprehensively described and analyzed is *G. cornuta*, which occurs in the Pacific lowlands of Ecuador and Colombia, reaching the Caribbean slopes of Costa Rica across Panama. According to Duellman (1970), males of this rare species (as *G. ceratophrys*) vocalize from the canopy, and the call sounds like a loud "bop" reminiscent of the sound of uncorking a bottle of champagne. It consists of one to three notes emitted at long intervals, usually between 8–12 min. The note duration is approximately 0.08 sec, and the inter-note interval is 0.6 sec (when the call is composed by more than one note). The note consists of three harmonics of 0.8 kHz (dominant

frequency), 1.6 kHz, and 2.4 kHz and there is frequency modulation, with each note diminishing abruptly from the beginning to the end. Comparisons of the numerical parameters of Duellman (1970) and the accompanying audiospectrogram of the call of *G. cornuta* (Plate 36.1) with those of *G. guentheri* presented herein (Fig. 3) show that *G. cornuta* emits shorter notes (0.08 sec vs. 0.262 sec in *G. guentheri*), with a lower dominant frequency (0.8 kHz vs. 1.8 kHz) and a much more pronounced, descending frequency modulation. Duellman (2015) briefly described the call of some other species of the *G. longipes* group. The calls of *G. dendronastes* is a loud chuckle-like sound followed by three to four “clucks;” *G. helenae* generates a call of 12–15 monosyllabic notes in a period of about five sec, and the call is repeated every 20–30 min; and the call of *G. weinlandii* is characterized by an explosive “wrock” usually followed by one or two shorter notes, “rock-rock” (Duellman 2015). Thus, the call characteristics within the *G. longipes* group seem quite variable, with short “bop” or “wrock”-like calls, either followed by short notes or not, and there are also calls composed by a succession of many monosyllabic notes like that of *G. helenae*.

Unfortunately, analyses of *Gastrotheca* vocalizations remain too scarce to make sound comparisons that would be sufficient to detect general inter- and intra-species group patterns. However, some patterns in vocalization characters variation seem to be detectable among several groups. For example, Sinsch and Juraske (2006) found consistent differences in calls of members of the *G. plumbea* group in relation to those of the *G. marsupiata* group (although all these species are now included in the broader *G. marsupiata* group of Castroviejo et al. [2015]). In general, these authors found that members of the *G. marsupiata* group emitted long pulsed advertisement calls in contrast to the more erratic, shorter calls of some members of their *G. plumbea* group. They even found distinctive call features among two clades within such a *G. plumbea* group, supporting a previous phylogenetic hypothesis by Duellman and Hillis (1987) based on allozymes. The short, erratic calls with frequency modulation often described as “bop” or “wrock” might be distinctive of members of the *G. longipes* group, although more research on *Gastrotheca* bioacoustics is needed to confirm this.

Conclusions

In cases of vanishing anuran species, description of the calls can be extremely useful for detecting remnants of populations, but it is important to record and safely store this type of information in general sound archives. For this reason, the publication of the calls in web checklists, like *Frog Calls of the World* (<http://www.fonozoo.com>), allows the comparison of any new recordings or hearing events with existing recorded sounds. The information on acoustic signals of *Paruwrobates andinus* and

Gastrotheca guentheri provided in this study could be useful to researchers and the personnel of local nature reserves or conservation projects, in order to detect these species in nature once again. Several Andean anuran species once considered extinct have been rediscovered in recent years in the form of relict populations (e.g., Barrio-Amorós et al. 2020, and references therein). In particular, rare species inhabiting the forest canopy can be especially difficult to register visually, so they can remain unnoticed for long periods of time even when they are present, e.g., the Colombian large treefrog *Ecnomiohyla phantasmagoria* (Dunn, 1943) reported by Duellman and di Domenico (2020) after nearly 80 years without any records. This might be the case of *G. guentheri*, but the conspicuous call of the species described herein should be useful in detecting it. Thus, we remain hopeful that, someday, researchers will announce the re-discovery of one of the two species of anurans studied herein, which were once sadly considered to be “extinct forever.”

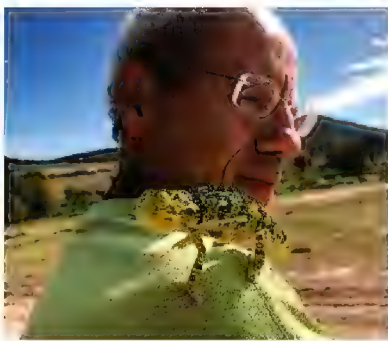
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Literature Cited

- Araya-Salas M, Smith-Vidaurre G, Webster M. 2019. Assessing the effect of sound file compression and background noise on measures of acoustic signal structure. *Bioacoustics* 28(1): 57–73.
- Arteaga A, Bustamante L, Guayasamin JM. 2013. *The Amphibians and Reptiles of Mindo. Life in the Cloudforest*. Universidad Tecnológica Indoamérica, Quito, Ecuador. 257 p.
- Barrio-Amorós CA, Costales M, Vieira J, Osterman E, Kaiser H, Arteaga A. 2020. Back from extinction: rediscovery of the Harlequin Frog, *Atelopus mindoensis* Peters, 1973, in Ecuador. *Herpetology Notes* 13: 325–328.
- Bauer L. 1994. New names in the family Dendrobatidae (Anura, Amphibia). *Ripa* [Den Haag] 1994(Fall): 1–6.
- Bosch J, De la Riva I, Márquez R. 2000. Advertisement calls of seven species of hyperoliid frogs from Equatorial Guinea. *Amphibia-Reptilia* 21(2): 246–255.
- Boulenger GA. 1882. *Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum. Second Edition*. Taylor and Francis, London, United Kingdom. 503 p.
- Burrowes PA. 1987. An ecological study of a cloud forest herpetofauna in southern Colombia. M.S.

- Thesis, Department of Systematics and Ecology, The University of Kansas, Lawrence, Kansas, USA.
- Castroviejo-Fisher S, Padial JM, De la Riva I, Pomal JP, da Silva HR, Rojas-Runjaic FJM, Medina-Méndez E, Frost DR. 2015. Phylogenetic systematics of egg-brooding frogs (Anura: Hemiphractidae) and the evolution of direct development. *Zootaxa* 4004: 1–75.
- Catenazzi A, Lehr E, Rodríguez LO, Vredenburg VT. 2011. *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. *Conservation Biology* 25(2): 382–391.
- Catenazzi A, Lehr E, Vredenburg VT. 2014. Thermal physiology, disease, and amphibian declines on the eastern slopes of the Andes. *Conservation Biology* 28: 509–517.
- Coloma LA. 1995. Ecuadorian frogs of the genus *Colostethus* (Anura: Dendrobatidae). *Miscellaneous Publications, Museum of Natural History, University of Kansas* 87: 1–72.
- Cornell Lab of Ornithology. 2014. Raven Pro: interactive sound analysis software. Version 1.5. Available: <http://ravensoundsoftware.com/software/raven-pro/> [Accessed: 20 March 2020].
- De la Riva I, Burrowes PA. 2019. Alarma en el paraíso: pasado, presente (¿y futuro?) de los anfibios en la reserva natural de La Planada, Colombia. *NaturalMente* 23: 55–62.
- Duellman WE. 1970. The hylid frogs of Middle America. *Monograph of the Museum of Natural History, the University of Kansas* 1: 1–753.
- Duellman WE. 2015. *Marsupial Frogs: Gastrotheca and Allied Genera*. John Hopkins University Press, Baltimore, Maryland, USA. 427 p.
- Duellman WE, Burrowes PA. 1989. New species of frogs, *Centrolenella*, from the Pacific versant of Ecuador and southern Colombia. *Occasional Papers of the Museum of Natural History, University of Kansas* 132: 1–14.
- Duellman WE, Hillis DM. 1987. Marsupial frogs (Anura: Hylidae: *Gastrotheca*) of the Ecuadorian Andes: resolution of taxonomic problems and phylogenetic relationships. *Herpetologica* 43: 141–173.
- Duellman WE, di Domenico S. 2020. Rediscovery of the Fantastic Treefrog, *Ecnomiohyla phantasmagoria* (Dunn) (Anura: Hylidae). *Herpetological Review* 51(2): 232–234.
- Duellman WE, Maxson LR, Jesiolowski CA. 1988. Evolution of marsupial frogs (Hylidae: Hemiphractinae): immunological evidence. *Copeia* 1988: 527–543.
- Edwards SR. 1971. Taxonomic notes on South American *Colostethus* with descriptions of two new species (Amphibia, Dendrobatidae). *Proceedings of the Biological Society of Washington* 84: 147–162.
- Frost DR. 2020. Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, New York, USA. Available: <https://amphibiansoftheworld.amnh.org/index.php> [Accessed: 15 April 2020].
- Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, et al. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297: 1–370.
- Gerhardt HC. 1994. The evolution of vocalization in frogs and toads. *Annual Review of Ecology and Systematics* 25: 293–324.
- Goicoechea N, De la Riva I, Padial JM. 2010. Recovering phylogenetic signal from frog mating calls. *Zoologica Scripta* 39(2): 141–154.
- Grant T, Humphrey EC, Myers CW. 1997. The median lingual process of frogs: a bizarre character of Old World ranoids discovered in South American dendrobatids. *American Museum Novitates* 3212: 1–40.
- Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299: 1–262.
- Grant T, Rada M, Anganoy-Criollo MA, Batista A, Dias PHS, Jeckel AM, Machado DJ, Rueda-Almonacid JV. 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology* 12(Special Issue): 1–90.
- Guillory WX, Muell MR, Summers K, Brown JL. 2019. Phylogenomic reconstruction of the Neotropical poison frogs (Dendrobatidae) and their conservation. *Diversity* 11(8): 126.
- IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-3. Available: <http://www.iucnredlist.org> [Accessed: 30 March 2020].
- IUCN SSC Amphibian Specialist Group. 2019a. *Ameerega andina*. The IUCN Red List of Threatened Species 2019: e.T55212A49338865.
- IUCN SSC Amphibian Specialist Group. 2019b. *Gastrotheca guentheri*. The IUCN Red List of Threatened Species 2019: e.T55338A85898956.
- Kahn TR, La Marca E, Lötters S, Brown JL, Twomey E, Amézquita A. (Editors). 2016. *Aposematic Poison Frogs (Dendrobatidae) of the Andean Countries: Bolivia, Colombia, Ecuador, Perú, and Venezuela*. Tropical Field Guide Series. Conservation International, Arlington, Virginia, USA. xxiii + 588 p.
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel M-O, Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods, and recommendations for best practice. *Zootaxa* 4251(1): 1–124.
- Lips KR. 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* 12: 106–117.
- Lips KR. 1999. Mass mortality and population declines of anurans at an upland site in western Panama.

- Conservation Biology* 13: 117–125.
- Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3,165–3,170.
- Lips KR, Diffendorfer J, Mendelson III JR, Sears MW. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* 6(3): e72.
- Lötters S, Jungfer K-H, Henkel FW, Schmidt W. 2007. *Poison Frogs. Biology, Species, and Captive Husbandry*. Edition Chimaira, Frankfurt am Main, Germany. 668 p.
- Lynch JD, Burrowes PA. 1990. The frogs of the genus *Eleutherodactylus* (family Leptodactylidae) at the La Planada Reserve in southwestern Colombia, with descriptions of eight new species. *Occasional Papers of the Museum of Natural History, University of Kansas* 136: 1–31.
- Muñoz-Arcos R, Guerrero-Cupacán JA, Cepeda-Quilindo B. 2016. First record of *Pristimantis crucifer* Boulenger, 1899 (Anura: Craugastoridae) from Colombia, Nariño Department. *Check List* 12(6): 2,021.
- Myers CW, Burrowes PA. 1987. A new poison frog (*Dendrobates*) from Andean Colombia, with notes on a lowland relative. *American Museum Novitates* 2899: 1–17.
- Myers CW, Ibáñez R, Grant T, Jaramillo CA. 2012. Discovery of the frog genus *Anomaloglossus* in Panama, with descriptions of two new species from the Chagres Highlands (Dendrobatoidea: Aromobatidae). *American Museum Novitates* 3763: 1–19.
- Paluh DJ, Stanley EL, Blackburn DC. 2019. First dietary record of *Gastrotheca guentheri* (Boulenger, 1882), the lone anuran with true mandibular teeth. *Herpetology Notes* 12: 699–700.
- Pisso GA, Silva ML, Maya AM, Vanegas J, Durán G. 2018. Lista preliminar de los anfibios del Parque Nacional Natural Munchique y áreas de influencia, departamento del Cauca-Colombia. *Revista*
- Novedades Colombianas* 13(1): 49–80.
- Scheele BC, Pasmans F, Berger L, Skerratt L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, et al. 2019. Amphibian fungal panzootic reveals catastrophic and ongoing loss of biodiversity. *Science* 363(6434): 1,459–1,463.
- Serrano-Rojas SJ, Whitworth A, Villacampa J, Von May R, Gutiérrez RC, Padial JM, Chaparro JC. 2017. A new species of poison-dart frog (Anura: Dendrobatidae) from Manu province, Amazon region of southeastern Peru, with notes on its natural history, bioacoustics, phylogenetics, and recommended conservation status. *Zootaxa* 4221(1): 71–94.
- Sinsch U, Juraske N. 2006. Advertisement calls of hemiphractine marsupial frogs: II. *Gastrotheca plumbea* group. Pp. 149–152 In: *Herpetologia Bonnensis II. Proceedings of the 13th Congress of the Societas Europaea Herpetologica*. Editors, Vences M, Köhler J, Ziegler T, Böhme W. Societas Europaea Herpetologica, Bonn, Germany. 262 p.
- Smith-Patten BD, Bridge ES, Crawford PH, Hough DJ, Kelly JF, Patten MA. 2015. Is extinction forever? *Public Understanding of Science* 24(4): 481–495.
- Sueur J, Aubin T, Simonis C. 2008. Equipment review: Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18: 218–226.
- Vigle GO, Miyata K. 1980. A new species of *Dendrobates* (Anura: Dendrobatidae) from the lowland rain forests of western Ecuador. *Breviora* 459: 1–7.
- Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105(Supplement 1): 11,466–11,473.
- Wiens JJ. 2011. Re-evolution of lost mandibular teeth in frogs after more than 200 million years, and re-evaluating Dollo's Law. *Evolution* 65: 1,283–1,296.
- Wiens JJ, Kuczynski CA, Duellman WE, Reeder TW. 2007. Loss and re-evolution of complex life cycles in marsupial frogs: does ancestral trait reconstruction mislead? *Evolution* 61(8): 1,886–1,899.



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Calls of two extirpated Colombian frogs



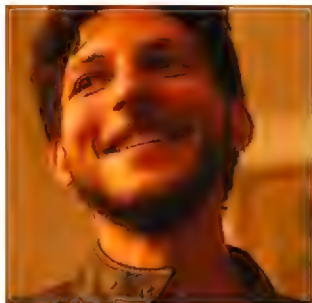
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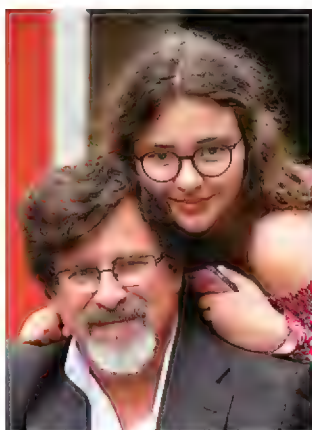
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Alien populations of painted frogs, genus *Discoglossus*, on the southeastern coast of France: two examples of anthropogenic introduction

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Abstract.—Introductions of animals and plants by humans permanently restructure the distribution ranges of species and the compositions of communities, a phenomenon which has been intensified in recent decades with globalization. However, it is often difficult to date these introductions or to identify the geographic origin of the introduced individuals. In this study, genetic variation in the mitochondrial gene for cytochrome *b* was examined in native populations of painted frogs (genus *Discoglossus*) and introduced individuals discovered at two novel locations in the south-east of France, to determine their specific ranks and origins. The population of *Discoglossus sardus* identified at Marseille probably originated from Corsica, and that of *Discoglossus pictus* discovered at Grimaud in the Var Department probably originated from the previously introduced range of the species in the southwestern Mediterranean region of France. These newly discovered populations of painted frogs represent an unresolved conservation issue, as they are allochthonous in the respective regions on one hand, but on the other hand they belong to species which are legally protected in France and Europe. As next steps, assessing their range expansion is important, as is studying the nature of the relationship between these painted frog populations and the native amphibian communities.

Keywords. Anura, biogeography, conservation, human introduction, invasive capacity, native range

Résumé.—Les introductions humaines d'animaux et de plantes restructurent en permanence l'aire de répartition des espèces et la composition des communautés, phénomène qui s'est intensifié ces dernières décennies avec la mondialisation. Cependant, il est souvent difficile de dater ces introductions et d'identifier leurs origines. Dans cette étude, la variation génétique du gène mitochondrial du cytochrome *b* a été examinée dans des populations indigènes de discoglosses (genre *Discoglossus*) et chez des individus introduits, découverts sur deux localités inédites dans le sud-est de la France. L'objectif étant de déterminer le rang spécifique et l'origine des discoglosses observés sur ces nouvelles localités. Les analyses témoignent de la présence de *Discoglossus sardus* (probablement originaire de Corse) à Marseille et de *Discoglossus pictus* (provenant probablement de l'aire d'introduction de l'espèce située dans le sud-ouest de la région méditerranéenne française) à Grimaud dans le département du Var. Ces populations nouvellement découvertes représentent un problème de conservation non résolu, car elles sont d'une part allochtones dans les localités respectives, mais d'autre part appartiennent à des espèces légalement protégées en France et en Europe. À l'avenir, il sera important d'évaluer leur expansion géographique et d'étudier la nature de la relation entre ces populations de discoglosses et les communautés d'amphibiens indigènes.

Mots clés. Anoure, biogéographie, conservation, introduction humaine, capacité d'invasion, aire de répartition d'origine

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Introduction

The presence of barriers, both natural (e.g., rivers, sea, mountains) and artificial (e.g., roads, urban centers), often limits the dispersal of terrestrial vertebrates (Peres et al. 1996; Epps et al. 2005; Riley et al. 2006; Delaney et al. 2010; Chiari et al. 2012). However, by their historic and contemporary activities, such as transport, international trade, experiments, agriculture, etc., humans have caused the introduction of species into territories far from their original distribution (Pyšek et al. 2010). This phenomenon has been exacerbated in recent decades due to globalization, which has intensified terrestrial, aerial, and sea transport, and increased international trade (Levine and D’Antonio 2003; Westphal et al. 2008; Hulme 2009). Many organisms, including amphibians, are affected by these anthropic introductions (either accidental or voluntary), which are often characterized by high potential population growth rates, allowing the introduced species to become permanently established (Kraus 2015; Aellen et al. 2017).

In France, numerous non-native amphibian species have settled successfully. Among the more historical introductions (i.e., in the early 20th century) are *Discoglossus pictus* in Banyuls-sur-Mer (Pyrénées-Orientales) and probably *Triturus carnifex*, which was introduced in Chêne-Bourg (Switzerland) [Lescure and de Massary 2012] very near the border with France and is now present in Ain and Haute-Savoie, around Lemane Lake (Arntzen 2001; Dufresnes et al. 2016). During the mid-20th century, *Pelophylax bergeri* was translocated from Central Italy multiple times, resulting in introgressive hybridization with native populations of its sister taxon *Pelophylax lessonae* (Dufresnes et al. 2017). During this time, massive importations of several other *Pelophylax* species (e.g., *P. bedriagae*) for human consumption also occurred (Pagano et al. 2003).

More recently, *Lithobates catesbeianus* was introduced in Arveyres (Gironde), *Xenopus laevis* in Bouillé-Saint-Paul (Deux-Sèvres), *Bombina bombina* in Albestroff (Moselle), and *Eleutherodactylus johnstonei* in the urban zone of Nantes (Loire-Atlantique) [Lescure and de Massary 2012; Labadesse and Eggert 2018]. In

addition, some indigenous species have been translocated within France, such as *Hyla meridionalis* into Hyères Islands (Knoepffler 1961), *Ichthyosaura alpestris* on the limestone plateau of Larzac (Hérault) [Denoël 2005; Geniez and Cheylan 2012], and *Speleomantes strinatii* into a mine in the French Pyrénées (Ariège) [Lunghi et al. 2018] and a cave near Angles-sur-l’Anglin (Vienne) [Lucente et al. 2016].

This report documents two additional cases, based on observations of painted frogs (*Discoglossus*) between 2011 and 2018 at two continental localities in the south-east of France, in the city of Marseille (Bouches-du-Rhône Department), and in a plain and semi-urban zone in the locality of Grimaud (Var Department) [Table 1, Fig. 1]. These observations have generated strong interest because the localities are geographically distant from the documented ranges of the two species of *Discoglossus* known to be present in France (Fig. 1). *Discoglossus sardus* is distributed in Sardinia, in the Tuscan Archipelago and the adjacent Italian coast, and in France in the eastern part of Hyères Islands (Port-Cros and the Levant Islands) and Corsica (Delaugerre and Cheylan 1992; Lescure and de Massary 2012). The other species, *Discoglossus pictus*, is indigenous to North Africa (Algeria and Tunisia), Sicily, Malta, and Gozo (Sindaco et al. 2006). However, since *D. pictus* was originally introduced into France in the department of Pyrenees-Orientales, it has colonized the adjacent departments of Aude, Hérault (Knoepffler 1962; Fradet and Geniez 2004; Geniez and Cheylan 2012), and the extreme north-east of Spain (Franch et al. 2007).

Because of the difficulty in unambiguously identifying species of *Discoglossus* by morphological criteria alone, molecular phylogenetic analyses were conducted to assess the species identity and geographic origins of the observed painted frogs from the two novel locations in mainland France.

Materials and Methods

Genetic samples. Tissue samples and buccal swabs were taken on 31 May 2018 and 17 June 2018, respectively, from nine tadpoles from Marseille; and on 7 November

Table 1. Available data on the occurrences of the two introduced species of *Discoglossus* on the southeastern coast of France. Ind. = Indeterminate.

Species	Date	Locality	Latitude (N)	Longitude (E)	Number of specimens	Observers
<i>Discoglossus sardus</i>	17 June 2011	Marseille	43°20'47.3"	5°26'10.9"	3–4	A. Piquet
	17 June 2015	Marseille	43°20'46.5"	5°26'22.4"	1	V. Mariani
	31 March 2018	Marseille	43°20'20.9"	5°26'32.4"	3	M. Policain
	16 April 2018	Marseille	43°21'00.7"	5°26'12.5"	60	M. Policain and F. Grimal
<i>Discoglossus pictus</i>	2016–2017	Grimaud	43°16'32.2"	6°31'52.9"	Ind.	V. Fradet and A. Dubois
	2016–2017	Grimaud	43°16'59.0"	6°30'59.8"	Ind.	V. Fradet and A. Dubois
	2016–2017	Grimaud	43°16'12.7"	6°33'02.6"	Ind.	V. Fradet and A. Dubois
	7 November 2018	Grimaud	43°15'52.6"	6°33'39.1"	3	J. Renet, M. Policain, and M. Marmier

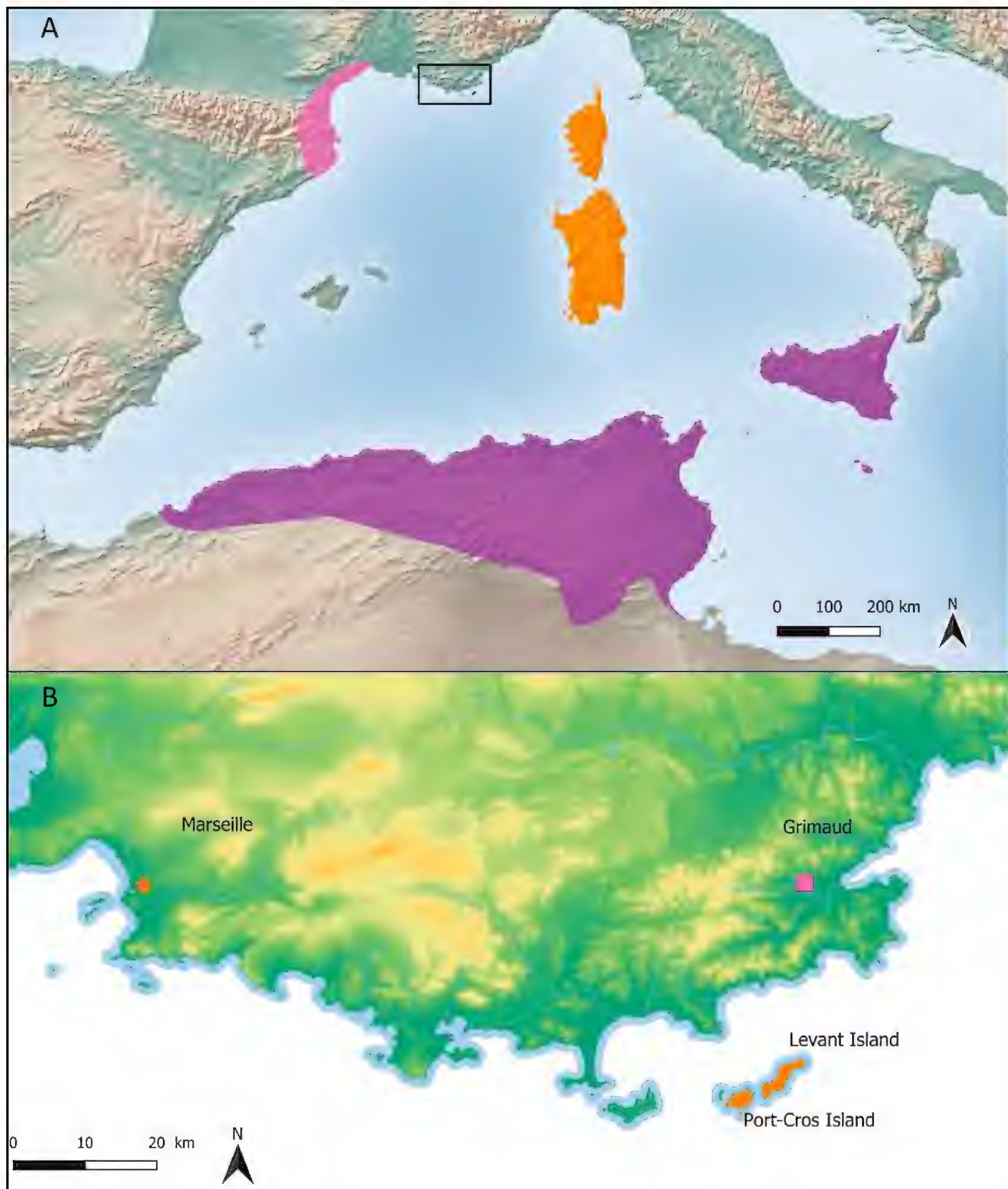


Fig. 1. (A) Map of the ranges of *D. sardus* (orange: native population range) and *D. pictus* (purple: original distribution range; pink: introduced population range). (B) Enlarged view of the area with the newly introduced populations of the two species in southern France (orange star: *D. sardus* in Marseille; pink square: *D. pictus* in Grimaud), which is indicated by the black rectangle in (A).

2018 from two adult specimens from Grimaud (Fig. 2C). The sampled individuals were collected at night along two small shady streams and in a water-filled moat bordering a wasteland and a vineyard (Fig. 2B,D). Additional comparative samples were collected from various sites in Sardinia, Corsica, and the Hyères Archipelago (Port-Cros), in the form of either muscle tissue samples from roadkill specimens or tail tips of tadpoles.

Genetic analyses. Total genomic DNA was extracted from the buccal swabs and tissue samples using a salt extraction protocol (Bruford et al. 1992). A fragment of the mitochondrial gene for cytochrome *b* (cob) was amplified using the primers in Zangari et al. (2006): MVZ15-L

(GAACTAATGGCCCACACWWTACGNAA) and H15149-H (AAACTGCAGCCCCTCAGAATGATATTGTCCTCA). As these primers did not reliably amplify the respective fragment, particularly in *D. sardus*, most samples were also amplified using two newly developed specific primers: Dsard-Fwd (TGACCTACCTACCCCATCCA) and Dsard-Rev (GGGCAGTACGTAGCCTACAA). For both primer pairs, the PCR protocol consisted of an initial step of 90 sec at 94 °C, followed by 35 steps of 94 °C (30 sec), 53 °C (45 sec), 72 °C (90 sec), and a final elongation step of 10 min at 72 °C. PCR products were treated with exonuclease I (New England Biolabs) and shrimp alkaline phosphatase (Promega) to inactivate remaining



Fig. 2. (A) Adult male *Discoglossus sardus* from Marseille, 31 May 2018. (B) *Discoglossus sardus* habitat in the city of Marseille. (C) Adult *Discoglossus pictus* from Grimaud, 7 November 2018. (D) *Discoglossus pictus* habitat in Grimaud. The white arrow indicates the position of a ditch filled with water, where three individuals were observed. Photos by Mathieu Policain (A–B), Julien Renet (C), and Google Map/Street View (D).

primers and dNTPs, and then sent for sequencing to LGC Genomics (Berlin, Germany). Chromatographs were checked and obvious errors in automated sequence reads were corrected using Codon-Code Aligner (v2.0.6, Codon Code Corporation). All newly determined sequences were submitted to GenBank (accession numbers MT569346–MT569387).

The cytochrome *b* fragment used was chosen to allow comparisons with the results of Zangari et al. (2006), who published sequences of *D. sardus* and all other species in the genus from various localities. These sequences were downloaded from GenBank and trimmed to match the shorter length of the sequences produced using the specific *D. sardus* primer pairs listed above. Note that this fragment is not homologous with the one used by Martínez-Solano (2004) and Vences et al. (2014), and therefore direct comparisons with the results of those studies (which focused on *D. galganoi* and *D. pictus*) are not possible.

Sequences were aligned and phylogenetic analysis was conducted using MEGA, v. 7 (Kumar et al. 2016). The sequences were first aligned using the Clustal algorithm, then the appropriate substitution model (Kimura-2-parameter + G) was selected under the Akaike Information Criterion, phylogenetic trees were subsequently inferred under the Maximum Likelihood

(ML) optimality criterion with NNI branch swapping, and node support was assessed with 500 bootstrap replicates. The tree was rooted with *D. montalentii*, which represents the sister species to all other *Discoglossus* (Zangari et al. 2006; Pabijan et al. 2012; Biton et al. 2013; Dufresnes et al. 2020).

Results and Discussion

Genetic Identity of the New *Discoglossus* Populations

The Maximum Likelihood tree reconstructed from the 258 bp cytochrome *b* segment retained for analysis (Fig. 3) recovered phylogenetic relationships among *Discoglossus* species which were largely similar to those of more comprehensive, multi-gene studies (Zangari et al. 2006; Pabijan et al. 2012; Biton et al. 2013; Dufresnes et al. 2020). However, as expected from such a short gene fragment, the relationships among most species were not reliably resolved. All species of *Discoglossus* were recovered as monophyletic groups, with bootstrap supports of 90–99%.

For the new continental French populations that are the focus of the present study, the tree is unambiguous in placing the samples from Marseille into the *D. sardus* clade, and the samples from Grimaud into the *D. pictus*

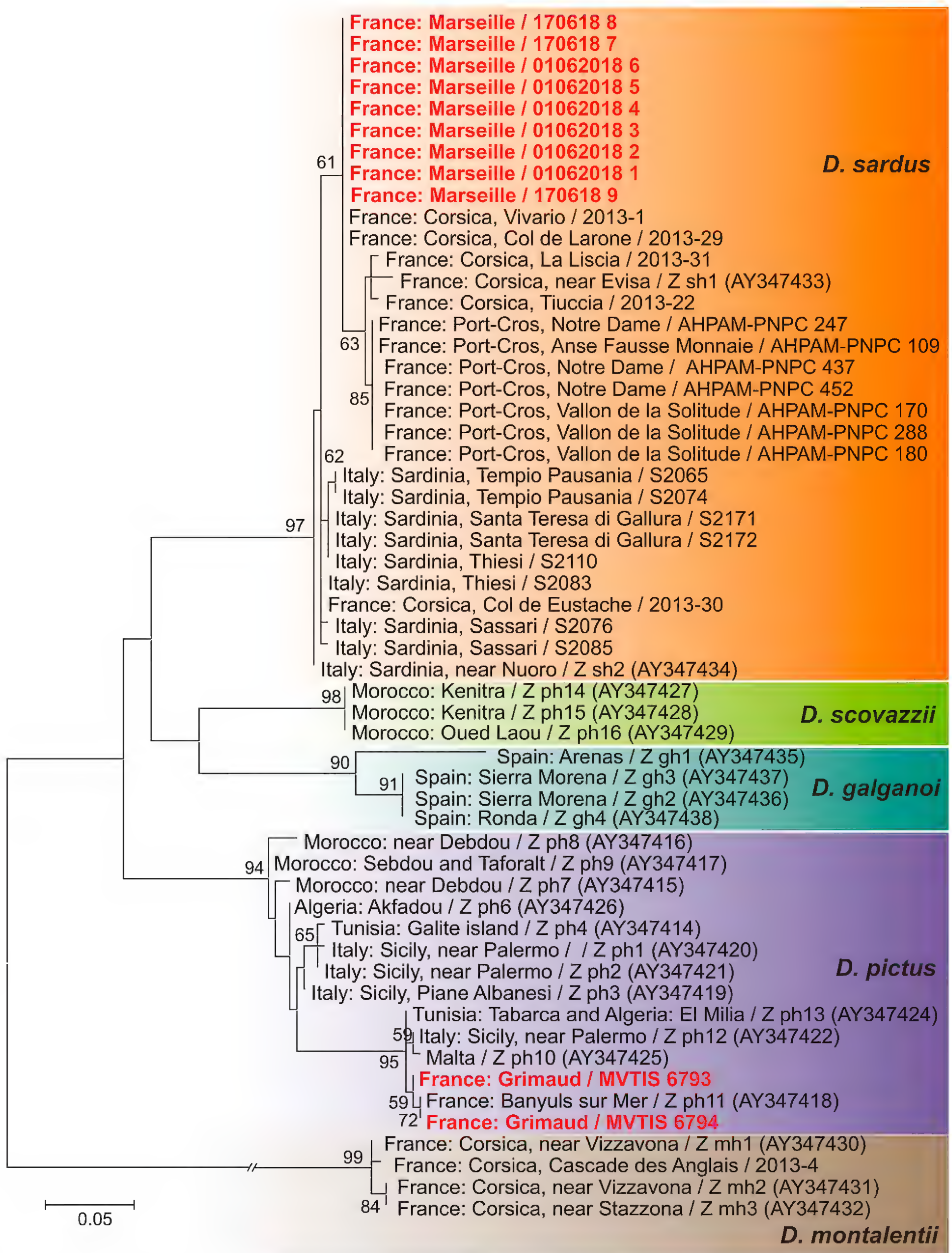


Fig. 3. Maximum Likelihood tree of *Discoglossus* based on a 258 bp fragment of the mitochondrial cytochrome *b* gene. Numbers at nodes are bootstrap values (500 pseudoreplicates) in percent. After the locality, sample numbers are given, including GenBank accession numbers in parentheses for those sequences taken from GenBank. “Z” marks sequences from the work of Zangari et al. (2006). Samples from the two newly discovered introduced populations are highlighted in bold, red font.

clade. All nine *D. sardus* specimens sequenced from Marseille had identical sequences, and the same haplotype was also found in two localities in Corsica. In contrast, all sequenced specimens from the Hyères Archipelago and from Sardinia differed by at least three mutations, suggesting that the Marseille population most likely originated by the introduction of only a few individuals from Corsica. Of the two specimens from Grimaud, one had a haplotype identical to that of a specimen from Banyuls-sur-Mer, while the second one differed by a single mutation. This suggests a probable origin of this population by introduction from the invasive range of *D. pictus* in the southwestern French Mediterranean region.

The tree generated here also recapitulates the surprising finding of Zangari et al. (2006) regarding the presence of rather distinct mitochondrial haplotypes of *D. pictus* in Sicily. It also reveals that *D. sardus* from Corsica and Sardinia are not reciprocally monophyletic based on mitochondrial DNA. In this latter case, the one Corsican *D. sardus* (from Col d'Eustache) clustering among the Sardinian haplotypes was sequenced several months before the samples from Sardinia were processed, which excludes the possibility of an artifact due to a mislabelled sample or contamination.

Geographic Origin and Status of the New *Discoglossus* Populations

The results of this analysis shed a new light on the ranges of the two species of *Discoglossus* that are present in the south of France. In the population of *D. sardus* established in Marseille represents the second known mainland population, after the population of Monte Argentario peninsula (Tuscany, Italy). The genetic similarity of the Marseille samples with those from the two Corsican localities allow us to refute the hypothesis of an ancient relict population naturally occurring in Marseille. In such a case we would expect genetic relationships with the individuals from Port-Cros or the Levant islands (Hyères Archipelago), which are geographically much closer to Marseille than Corsica. On the contrary, an introduction from Corsica is consistent with the intensity of the maritime traffic between Corsica and Marseille, and the apparent lack of genetic diversity in the Marseille samples is also in agreement with an introduced origin. Even if the date of introduction of this species cannot be determined, its spread over an area of approximately 0.66 km² suggests that the arrival of *D. sardus* in Marseille is not very recent.

With a geographic extension of almost 210 km to the east (i.e., the distance between the easternmost population known to date and the recently discovered population at Grimaud), and its crossing of the Rhone River, the anthropic introduction of *D. pictus* is beyond doubt. Its arrival in Grimaud, along the La Garde river, could be linked with the trade activities of the many nurseries and garden stores (12 shops identified within

10 km of the sampling locality), which are known to be vectors of various species introductions worldwide (e.g., anurans, snails, plants; Christy et al. 2007; Bergey et al. 2014).

The two newly detected introductions of *Discoglossus* in continental France could have been accidental, or they could have been deliberate due to a variety of motivations, such as experimental studies on naturalization conducted in the past, or the liberation of captive animals. For instance, *D. sardus* tadpoles from Port-Cros Island were introduced into a tributary of la Mole river (Var) as an experiment in 1955, and this attempt at establishing a reproducing population is known to have succeeded at least until 1959 (Knoepffler 1962).

The discoveries of these new populations testify once again that today the natural elements, such as rivers or oceans, do not represent absolute barriers for either native or allochthonous species. Invasion success generally depends more on the ability of a species to respond to natural selection than on broad physiological tolerance or plasticity (Lee 2002). In the present case, considering the ranges of these two species and their reproductive status, it seems that they can be considered as successful colonizers. In fact, more comprehensive phylogeographic studies of *D. pictus* and *D. sardus* in the future should also examine the possibilities of *D. sardus* translocations among Corsica and Sardinia (given the clustering of the one Corsican haplotype among the Sardinian haplotypes; Fig. 3) and of *D. pictus* to or from Sicily (given the presence of highly distinct haplotypes on this island; Fig. 3).

Conservation Issues

Williamson (1996) considers that a biological invasion occurs when an organism takes root outside of its indigenous range. The IUCN Invasive Species Specialist Group proposes a more specific definition—that a biological invasion has occurred as soon as an introduced species is a factor of damage and affects the local biodiversity. In fact, it is important to distinguish between an allochthonous species introduced by humans, which is inoffensive in many cases, and an invasive species, which, by definition, is not only introduced outside of its indigenous range but also exerts a negative impact on biodiversity and more globally on the ecosystem (Lambertini et al. 2011).

In the urban and sub-optimal ecological context of the city of Marseille, the population of *D. sardus* probably does not represent a threat to the ecosystem, which is *a priori* of 'low ecological value.' Furthermore, this population is already threatened by a large-scale urban development project. Although *D. sardus* is considered to be Least Concern in both the IUCN Red List and the National French Red List (Andreone et al. 2009; UICN France et al. 2015), the global assessment has determined a decreasing population trend. This points to an important

and challenging dilemma highlighted by Marchetti and Engstrom (2016): how to manage allochthonous, or even invasive species, that are threatened (or may become threatened in the future) in their native range? Several authors (e.g., Marris 2014; Heise 2018) have suggested pragmatic approaches when dealing with non-native species, especially in urban environments which indeed could become sanctuaries for many species (native or not) that are threatened in their original habitat. Especially with shifting ranges due to climatic change, the distinctions between native and non-native will become increasingly vague, and human-aided translocations of some threatened species are already being discussed (Egan et al. 2018).

These elements lead us to consider the presence of these new *Discoglossus* populations as a high-priority conservation issue. We can also add that *D. sardus* is assessed as Threatened in the Var Department (cat. VU IUCN Redlist) [Marchand et al. 2017], and as threatened with extinction at Port-Cros Island, Port-Cros National Park (Duguet et al. 2019).

Concerning *D. pictus*, the question of its biological status requires more scrutiny because other authors have attributed an invasive nature with a high rate of dispersal to this species (Montori et al. 2007). Its invasive capacity does not seem to be related to its adaptive advantages, but rather to the suitability of local abiotic conditions (Escoriza et al. 2014). The modeling of its potential habitat conducted by Escoriza et al. (2014) includes areas that are geographically near the locality of Grimaud, and incorporation of the new occurrences should allow an adjustment of the predictive models. Furthermore, the potential area of this species should be considered as wider than suggested by previous models. In any case, the expansion of *D. pictus* from a single location in Banyuls-sur-Mer, Eastern Pyrénées a century ago (see Wintrebert 1908) is not an artifact; i.e., it represents a natural range expansion (Pujol-Buxó et al. 2019a) into a currently occupied area in France and Catalonia of more than 10,000 km² (Montori et al. 2009). A negative impact of this species on co-occurring anurans (e.g., *Pelodytes punctatus* and *Epidalea calamita*) has been suspected (Escoriza and Boix 2012, 2014; Richter-Boix et al. 2013; San Sebastián et al. 2015). However, this possibility requires further study as some have hypothesized that temporal or evolutionary changes may have moderated the effects and disturbance of *D. pictus* on native species (Pujol-Buxó et al. 2019b).

In any event, according to the actual current French regulations, all individuals of both species, as well as their “core” habitat, are strictly protected by a ministerial order (DEVN0766175A). Although a recent update of this order would specifically exclude *D. pictus*, we hope for the continued regulatory protection of *D. pictus* in French territory. Given the similarities in biotic features between the source and recipient communities (Escoriza and Ruhí 2016), we suspect that *Discoglossus* species

are probably not harmful to the local French anuran communities, and we therefore do not recommend the eradication of their non-native populations.

Lastly, to better manage this situation going forward, we recommend a monitoring program to: (1) characterize a predictable range expansion of these two painted frog species in adjacent localities; and (2) implement complementary studies in order to better assess the nature of the relationship between these introduced species and the native amphibian communities.

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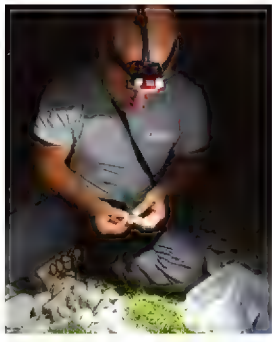
Literature Cited

- Aellen WL, Street SE, Capellini I. 2017. Fast life history traits promote invasion success in amphibians and reptiles. *Ecology Letters* 20: 222–230.
- Andreone F, Lecis R, Miaud C, Corti C, Sindaco R, Romano A. 2009. *Discoglossus sardus*. The IUCN Red List of Threatened Species 2009: e.T55271A11265832.
- Arntzen JW. 2001. Genetic variation in the Italian Crested Newt, *Triturus carnifex*, and the origin of a non-native population north of the Alps. *Biodiversity and Conservation* 10: 971–987.
- Bergey EA, Figueroa LL, Mather CH, Martin, RJ, Ray EJ, Kurien JT, Westrop DR, Suriyawong P. 2014. Trading in snails: plant nurseries as transport hubs for non-native species. *Biological Invasions* 16: 1,441–1,451.
- Biton R, Geffen E, Vences M, Cohen O, Bailon S, Rabinovich R, Malka Y, Oron T, Boistel R, Brumfeld V, et al. 2013. The rediscovered Hula Painted Frog is a living fossil. *Nature Communications* 4: e1959.
- Bruford MW, Hanotte O, Brookfield JFY, Burke T. 1992. Single-locus and multilocus DNA fingerprint. Pp. 225–270 In: *Molecular Genetic Analysis of Populations: a Practical Approach*. Editor, Hoelzel AR. IRL Press, Oxford, United Kingdom. 445 p.
- Chiari Y, van der Meijden A, Mucedda M, Lourenço JM, Hochkirch A, Veith M. 2012. Phylogeography of Sardinian cave salamanders (genus *Hydromantes*) is mainly determined by geomorphology. *PLoS One* 7(3): e32332.
- Christy MT, Savidge JA, Rodda GH. 2007. Multiple pathways for invasion of anurans on a Pacific island. *Diversity and Distributions* 13: 598–607.
- Delaney KS, Riley SP, Fisher RN. 2010. A rapid, strong, and convergent genetic response to urban habitat

- fragmentation in four divergent and widespread vertebrates. *PLoS One* 5(9): e12767.
- Delaugerre M, Cheylan M. 1992. *Atlas de répartition des batraciens et reptiles de Corse*. L'Oikéma, Pamplona, Spain. 128 p.
- Denoël M. 2005. Persistence et dispersion d'une population introduite de Triton alpestre (*Triturus alpestris*) dans les causses du Larzac (Sud de la France). *Revue d'Écologie (Terre et Vie)* 60: 139–148.
- Dufresnes C, Pellet J, Bettinelli-Riccardi S, Thiebaud J, Perrin N, Fumagalli L. 2016. Massive genetic introgression in threatened Northern Crested Newts (*Triturus cristatus*) by an invasive congener (*T. carnifex*) in Western Switzerland. *Conservation Genetics* 17: 839–846.
- Dufresnes C, Di Santo L, Leuenberger J, Schuerch J, Mazepa G, Grandjean N, Canestrelli D, Perrin N, Dubey S. 2017. Cryptic invasion of Italian Pool Frogs (*Pelophylax bergeri*) across Western Europe unraveled by multilocus phylogeography. *Biological Invasions* 19: 1,407–1,420.
- Dufresnes C, Pribille M, Alard B, Gonçalves H, Amat F, Crochet PA, Dubey S, Perrin N, Fumagalli L, Vences M, et al. 2020. Integrating hybrid zone analyses in species delimitation: lessons from two anuran radiations of the Western Mediterranean. *Heredity* 124: 423–438.
- Duguet R, Priol P, Deso G, Geoffroy D. 2019. Mise à jour des connaissances sur le Discoglosse sarde *Discoglossus sardus* Tschudi in Otth, 1837 dans l'île de Port-Cros en 2018: habitats potentiels, état de la population et mesures de gestion. *Scientific Reports of Port-Cros National Park* 33: 101–126.
- Egan PA, Bourke D, Thuiller W, Baudraz MEA, Georges D, Renaud J, Stout JC. 2018. Invasive aliens threatened with native extinction: examining best practice for species translocations under climate change. bioRxiv preprint. Available: <https://doi.org/10.1101/429084> [Accessed: 15 March 2020].
- Epps CW, Palsbøll PJ, Wehausen JD, Roderick GK, Ramey RRII, McCullough DR. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert Bighorn Sheep. *Ecology Letters* 8: 1,029–1,038.
- Escoriza D, Boix D. 2012. Assessing the potential impact of an invasive species on a Mediterranean amphibian assemblage: a morphological and ecological approach. *Hydrobiologia* 680: 233–245.
- Escoriza D, Boix D. 2014. Reproductive habitat selection in alien and native populations of the genus *Discoglossus*. *Acta Oecologica* 59: 97–103.
- Escoriza D, Ben Hassine J, Boix D. 2014. Factors regulating the invasive success of an alien frog: a comparison of the ecology of the native and alien populations. *Hydrobiologia* 730: 127–138.
- Escoriza D, Ruhí A. 2016. Functional distance to recipient communities may favor invasiveness: insights from two invasive frogs. *Diversity and Distributions* 22: 519–533.
- Fradet V, Geniez P. 2004. La répartition du Discoglosse peint *Discoglossus pictus* Otth, 1837 (Amphibien, Anoures, Discoglossidés) dans le Sud de la France: note sur sa présence dans le département de l'Hérault. *Bulletin de la Société Herpétologique de France* 109: 35–41.
- Franch M, Llorente GA, Montori A, Richter-Boix A, Carranza S. 2007. Discovery of an introduced population of *Discoglossus pictus* beyond its known distributional range. *Herpetological Review* 38: 356–359.
- Geniez P, Cheylan M. 2012. *Les Amphibiens et les Reptiles du Languedoc-Roussillon et régions limitrophes: atlas biogéographique*. Muséum national d'Histoire naturelle, Paris, France and Biotope, Mèze, France. 448 p.
- Heise UK. 2018. The case for 'sanctuary cities' for endangered species. Available: <https://www.citylab.com/environment/2018/06/the-case-for-sanctuary-cities-for-endangered-species/562091/> [Accessed: 15 March 2020].
- Hulme PE. 2009. Trade, transport, and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46(1): 10–18.
- Knoepffler LP. 1961. Les Batraciens et principalement le genre *Discoglossus* dans les îles méditerranéennes. *Colloques internationaux du Centre National de la Recherche Scientifique* 94: 159–161.
- Knoepffler LP. 1962. Contribution à l'étude du genre *Discoglossus* (Amphibiens Anoures). *Vie et Milieu* 13: 1–94.
- Kraus F. 2015. Impacts from invasive reptiles and amphibians. *Annual Review of Ecology, Evolution, and Systematics* 46: 75–97.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1,870–1,874.
- Labadesse M, Eggert C. 2018. La gestion intégrée des amphibiens exotiques envahissants en France métropolitaine. *Faune Sauvage* 321: 58–63.
- Lambertini M, Leape J, Marton-Lefèvre J, Mittermeier RA, Rose M, Robinson JG, Stuart SN, Waldman B, Genovesi P. 2011. Invasives: a major conservation threat. *Science* 333: 404–405.
- Lee CE. 2002. Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17(8): 386–391.
- Lescure J, de Massary JC. 2012. *Atlas des Amphibiens et Reptiles de France*. Biotope, Mèze, France and Muséum national d'Histoire naturelle, Paris, France. 272 p.
- Levine JM, D'Antonio CM. 2003. Forecasting biological invasions with increasing international trade. *Conservation Biology* 17: 322–326.
- Lucente D, Renet J, Gailledrat M, Tillet J, Nascetti G,

- Cimmaruta R. 2016. A new population of European cave salamanders (genus *Hydromantes*) from west-central France: relict or introduction? *Herpetological Bulletin* 138: 21–23.
- Lunghi E, Guillaume O, Blaimont P, Manenti R. 2018. The first ecological study on the oldest allochthonous population of European cave salamanders (*Hydromantes* sp.). *Amphibia-Reptilia* 39: 113–119.
- Marchand MA, Roy C, Renet J, Delauge J, Meyer D, Hayot C. 2017. *Liste rouge régionale des amphibiens et reptiles de Provence-Alpes-Côte d'Azur*. Conservatoire d'espaces naturels Provence-Alpes-Côte d'Azur, Aix-en-Provence, France. 14 p.
- Marchetti MP, Engstrom T. 2016. The conservation paradox of endangered and invasive species. *Conservation Biology* 30: 434–437.
- Marris E. 2014. Opinion: it's time to stop thinking that all non-native species are evil. *National Geographic Magazine*. Available: <http://news.nationalgeographic.com/news/2014/07/140724-invasive-species-conservation-biology-extinction-climate-science/> [Accessed: 9 August 2019].
- Martínez-Solano I. 2004. Phylogeography of Iberian *Discoglossus* (Anura, Discoglossidae). *Journal of Zoological Systematics and Evolutionary Research* 42: 298–305.
- Montori A, Llorente GA, Richter-Boix A, Geniez P, Villero D, San Sebastián O, Franch M, Garriga N. 2009. L'expansion de *Discoglossus pictus* pose t-elle des problèmes de coexistence pour les autres amphibiens? Oral presentation at: 37^{ème} Congrès de la Société herpétologique de France, 8–10 octobre 2009, Montpellier, France.
- Montori A, Llorente GA, Richter-Boix A, Villero D, Franch M, Garriga N. 2007. Colonización y efectos potenciales de la especie invasora *Discoglossus pictus* sobre las especies nativas. *Munibe* 25: 14–27.
- Pabijan M, Crottini A, Reckwell D, Irisarri I, Hauswaldt JS, Vences M. 2012. A multigene species tree for Western Mediterranean painted frogs (*Discoglossus*). *Molecular Phylogenetics and Evolution* 64: 690–696.
- Pagano A, Dubois A, Lesbarrères A, Lodé T. 2003. Frog alien species: a way for genetic invasion? *Comptes Rendus Biologies* 326: 85–92.
- Peres CA, Patton JL, Nazareth F, da Silva M. 1996. Riverine barriers and gene flow in Amazonian Saddle-back Tamarins. *Folia Primatologica* 67: 113–124.
- Pujol-Buxó E, Garcia Cisneros A, Miaud C, Llorente GA. 2019a. Genetic relationships and diversity patterns within the invasive range of the Mediterranean Painted Frog. *Journal of Zoology* 309: 125–132.
- Pujol-Buxó E, Riaño GM, Llorente GA. 2019b. Stable isotopes reveal mild trophic modifications in a native-invasive competitive relationship. *Biological Invasions* 21: 1,167–1,177.
- Pyšek P, Jarošík V, Hulme PE, Kühn I, Wild J, Arianoutsou M, Bacher S, Chiron F, Didžiulis V, Essl F, et al. 2010. Disentangling the role of environmental and human pressures on biological invasions across Europe. *Proceedings of the National Academy of Sciences of the United States of America* 107(27): 12,157–12,162.
- Richter-Boix A, Garriga N, Montori A, Franch M, San Sebastián O, Villero D, Llorente GA. 2013. Effects of the non-native amphibian species *Discoglossus pictus* on the recipient amphibian community: niche overlap, competition, and community organization. *Biological Invasions* 15: 799–815.
- Riley SP, Pollinger JP, Sauvaget RM, York EC, Bromley C, Fuller TK, Wayne RK. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology* 15: 1,733–1,741.
- San Sebastián O, Pujol-Buxó E, Garriga N, Richter-Boix A, Llorente GA. 2015. Differential trophic traits between invasive and native anuran tadpoles. *Aquatic Invasions* 10(4): 475–484.
- Sindaco R, Doria G, Razzetti E, Bernini F. 2006. *Atlas of Italian Amphibians and Reptiles - Atlante degli Anfibi e dei Rettili d'Italia*. Societas Herpetologica Italica - Edizioni Polistampa, Firenze, Italy. 789 p.
- UICN France, MNHN, SHF. 2015. *La Liste rouge des espèces menacées en France. Chapitre Reptiles et Amphibiens de France métropolitaine*. Union internationale pour la conservation de la nature France, Muséum national d'Histoire naturelle, and Société herpétologique de France, Paris, France. 7 p.
- Vences M, de Pous P, Nicolas V, Díaz-Rodríguez J, Donaire D, Hugemann K, Hauswaldt JS, Amat F, Barnestein JAM, Bogaerts S, et al. 2014. New insights on phylogeography and distribution of painted frogs (*Discoglossus*) in northern Africa and the Iberian Peninsula. *Amphibia-Reptilia* 35: 305–320.
- Westphal MI, Browne M, MacKinnon K, Noble I. 2008. The link between international trade and the global distribution of invasive alien species. *Biological Invasions* 10: 391–398.
- Williamson M. 1996. *Biological Invasions*. Chapman and Hall, London, United Kingdom. 256 p.
- Wintrebert P. 1908. Quinzième Assemblée Générale Annuelle, Séance du 25 février 1908. Intervention de M. Wintrebert sur la présence à Banyuls-sur-Mer (Pyrénées-Orientales) du *Discoglossus pictus* Otth. *Bulletin de la Société Zoologique de France* 33: 54.
- Zangari F, Cimmaruta R, Nascetti G. 2006. Genetic relationships of the western Mediterranean painted frogs based on allozymes and mitochondrial markers: evolutionary and taxonomic inferences (Amphibia, Anura, Discoglossidae). *Biological Journal of the Linnean Society* 87: 515–536.

Anthropogenic introduction of *Discoglossus* in southern France



Julien Renet is a French wildlife biologist at the NGO Conservatoire d'espaces naturels de Provence Alpes Côte d'Azur (CEN PACA, <http://www.cen-paca.org/>), where he designs, coordinates, and implements the conservation programs for several species of herpetofauna (e.g., *Pelobates cultripipes*, *Triturus cristatus*, *Emys orbicularis*, *Euleptes europaea*) of the Provence-Alps-Côte d'Azur region in France. His work concerns the general framework of conservation biology, more specifically ecology, biogeography, population dynamics, and assessments of non-invasive individual marking methods. Additional information about his work is available on his ResearchGate page, at: https://www.researchgate.net/profile/Julien_Renet/research.



Rémi Duguet is a private consultant for biodiversity assessments and monitoring, and a part-time teacher of herpetology at the University of Franche-Comté, Besançon, France. Rémi has worked for many years in amphibian and reptile conservation, especially in France and in the western Indian Ocean islands. He edited the book *Les Amphibiens de France, Belgique et Luxembourg*, published in 2003 by Biotope (Meze, France).



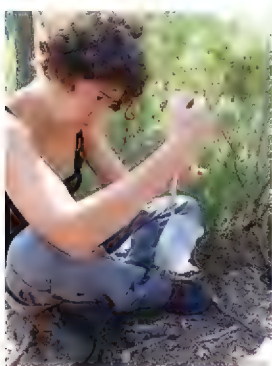
Mathieu Policain is a naturalist who has worked for the NGO Colinéo-Assenemce (Marseille, France, <https://colineo.fr/>) for five years. He has a special interest in the Mediterranean herpetofauna, and is currently an active volunteer in various efforts to protect amphibians and reptiles.



Alison Piquet is a French herpetologist with a Master's degree and almost ten years of national and international experience in the field. Specializing in reptiles, and snakes in particular, she travels extensively to conduct inventory field studies and observe reptiles, amphibians, birds, and spiders in the wild. As a herpetologist, she joined the *Radeau des Cimes* international expedition to Laos in 2014, in order to inventory the herpetofauna in a poorly-known primary forest area. She also spent a few years in Australia studying and photographing the amazing wildlife of that country.



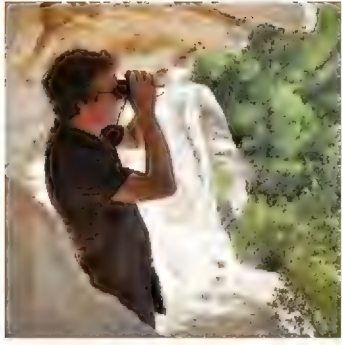
Vincent Fradet is an amphibian specialist who graduated from the École Pratique des Hautes Études (Paris-Sorbonne) where he studied *Discoglossus pictus* phylogeography. Vincent now works in the service of nature for several environmental NGOs.



Pauline Priol works as scientific consultant in conservation biology, has spent several years managing conservation programs for endangered species (*Emys orbicularis*, *Pelobates cultripipes*), and obtained two graduate degrees from universities in France and Canada. Pauline is now working with field practitioners, various stakeholders, and statisticians to develop methods for modeling population dynamics, building and evaluating monitoring protocols, estimating demographic parameters, evaluating impacts of perturbations, and evaluating/defining management actions. Her specialty is herpetofauna (e.g., European pond turtles *Emys* and *Mauremys*, crested newts, *Discoglossus*, spadefoot toads, Mediterranean lizards) but she also works on birds (stock programs, woodcock), crayfish, and insects (dragonflies, butterflies).



Grégory Deso is a herpetologist who has been active in environmental organizations since 1999 on the Mascarene Islands, where his work has focused on the distribution and ecology of various native and introduced species and their interactions linked to human activity. He now resides in mainland France (Provence Alps Côte d'Azur region) where he founded the NGO Association Herpétologique de Provence Alpes Méditerranée (<https://ahpam.fr/>), which works toward the protection of amphibians and reptiles. Today, Gregory's work concerns all aspects of the continental and island Mediterranean herpetofauna.



François Grimal is a French wildlife biologist at the NGO Ligue pour la Protection des Oiseaux (LPO, <https://www.lpo.fr/>), an affiliate of Birdlife International. François designs and coordinates conservation and monitoring programs for several amphibian populations of the Provence-Alps-Côte d'Azur region, in particular *Epidalea calamita* and *Pelophylax* sp. His work concerns the ecology, population dynamics, implementation of genetic and bioacoustic studies, and photographic and individual marking methods.



Giuseppe Sotgiu is an Italian biologist who works as an independent researcher specializing in the conservation of the insular herpetofauna and ichthyofauna of Sardinia. The species he primarily studies is the Sardinian Newt, *Euproctus platycephalus*, one of the most endangered urodeles in Europe. Giuseppe collaborates with the Department of Zoology of the University of Sassari, Sardinia, Italy. Since 2007, he has also collaborated with the Institute of Zoology at the Zoological Society of London, in order to understand the impacts of pathogens such as chytridiomycete fungi on the amphibian populations in the Mediterranean islands. He is also interested in studying the impacts of alien species on native endemic species, and developing methods to mitigate the effects of ecological invasions.



Miguel Vences is a zoologist and evolutionary biologist at Braunschweig University of Technology, Germany. He leads a long-standing research program on amphibian and reptile biology, with investigations spanning classical taxonomy, molecular evolution, diversification processes, biogeography, and conservation biology. Miguel has worked extensively on the herpetofauna of Madagascar, and also on numerous taxa in Europe.



On the Critically Endangered Cofre de Perote Salamander (*Isthmura naucampatepetl*): discovery of a new population in Puebla, Mexico, and update of its known distribution

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Abstract.—The presence of the Cofre de Perote Salamander, *Isthmura naucampatepetl*, in the state of Puebla, Mexico, is confirmed based on a population found during recent forestland surveys. The new population is the largest known for the species, including at least 26 individuals. Information about the size, weight, and color pattern variations is provided for this rarely seen species, and its distribution and conservation needs are briefly discussed.

Keywords. Amphibia, Caudata, confirmation, color pattern, lost species, Plethodontidae

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Introduction

Amphibians are one of the vertebrate groups in which the current mass extinction episode is most evident (Bishop et al. 2012). The Zoological Society of London identified 100 priority amphibian species as Evolutionarily Distinct and Globally Endangered (i.e., “EDGE species”), and the Amphibian Survival Alliance lists 34 Lost Species of amphibians (ZSL 2008; García-Bañuelos et al. 2017). Among salamanders, the IUCN lists 57 Mexican species as Critically Endangered (IUCN 2020), one of which is the Cofre de Perote Salamander (*Isthmura naucampatepetl*).

A member of the *Isthmura belli* complex (Parra-Olea et al. 2005), *I. naucampatepetl* was described based on five specimens, all of which were collected in 1981 on a narrow ridge extending east from Cofre de Perote and terminating in a small peak (Cerro Volcancillo) in the Sierra Madre Oriental of central Veracruz, México. The collecting locality was Cerro Las Lajas on the slopes of Cofre de Perote, and Cerro Volcancillo (Parra-Olea and Wake 2001; Parra-Olea et al. 2005; IUCN 2020). Recently, *I. naucampatepetl* was recorded at two localities in Puebla State, and those records were uploaded into the Naturalista, CONABIO portal ([https://www.naturalista.](https://www.naturalista.mx/)

[mx/search?q=Isthmura%20naucampatepetl](https://www.naturalista.mx/search?q=Isthmura%20naucampatepetl); Accessed: 1 September 2019). The photographs were taken on 6 October 2015 in the Municipality of Teziutlán, near the localities of San Juan Acateno and Atoluca, and published in the Naturalista website without precise locality information. Therefore, this vague record was not included in the most recent cataloging of the herpetofauna of Puebla (Woolrich et al. 2017).

Only very limited information on this species has been available thus far, based on only seven specimens from four localities. This article provides data from the largest known population of the species (26 individuals) which was found in a new locality in Puebla, Mexico, confirming the presence of *I. naucampatepetl* in this state. Herein we provide novel data on the size, weight, and color pattern variations of this enigmatic species, and briefly comment on relevant conservation implications.

Materials and Methods

Surveys were conducted on 20 forested lands of the Unidad de Manejo Forestal (UMAFOR 2103-Teziutlán) in the Sierra Norte of Puebla, including the zone Bienes Comunes San Mateo Chignautla, in the Municipality

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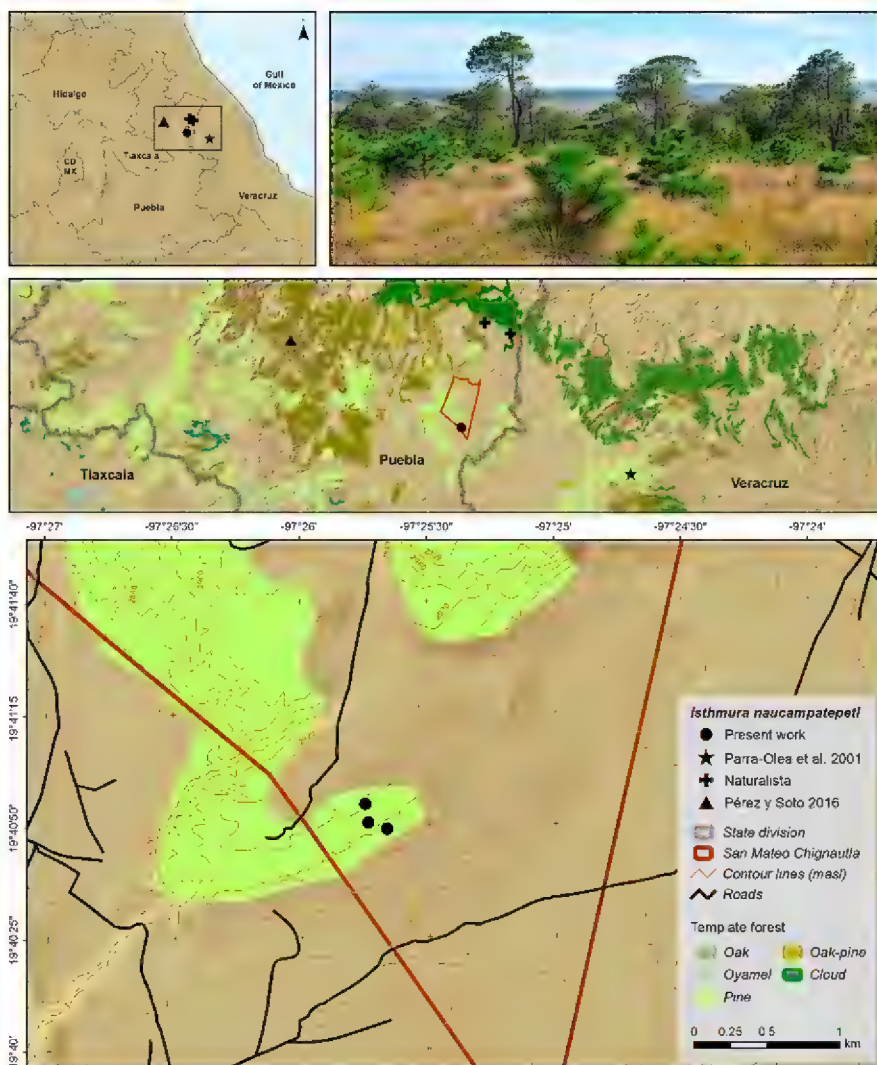


Fig. 1. Distribution maps of *Isthmura naucampatepetl*. Black circles represent published records, star represents the new population in the Municipality of Chignautla, Puebla, Mexico. The photograph shows the habitat at the new population. Photo by L. Fernández-Badillo.

of Chignautla, state of Puebla, México (Fig. 1). The zone Bienes Comunes has an area of 5,740.2 ha, of which 969.33 ha are earmarked for wood harvesting. The work presented here formed part of the project *Estudio regional de fauna en bosques de producción de la UMAFOR 2103 Teziutlán, Puebla, México* (WFC and ARST 2018). Field work was authorized by SEMARNAT permit SGPA/DGVS/07199/17.

All captured *I. naucampatepetl* individuals were measured, weighed, and photographed in the field, and subsequently released at the original collection site. Measurements of length were taken using a digital caliper, and weights were obtained using an electronic pocket scale (max. 500 g, min. 0.1 g). Length measurement abbreviations are as follows: SVL = snout-vent length; TL = tail length; and T = total length. A photograph of one salamander was deposited in the photographic collection of the Herpetological Collection of the Biological Research Centre of the Autonomous University of Hidalgo (CH-CIB).

A bibliographic review of the available information on this species was also performed. This review includes information from a record provided by Pérez y Soto (2016), who presented a photograph and data for some specimens identified as *I. gigantea*, but they actually correspond to *I. naucampatepetl*. Additional records in the Naturalista database (Naturalista CONABIO 2020) are also included. This information serves to comprehensively update the known distribution of this species.

Results

Field records. The first salamander was observed on 6 July 2017 (CH-CIB 117; Figs. 1,2a) under a rock in grassland habitat at ca. 1100 h (19.70668°N, -97.43773°W; WGS 84), elevation 2,879 m. On 16 September 2017, 25 additional salamanders were found in a grassland habitat with some reforested pine trees at around 1700 h (19.680720°N, -97.428987°W; WGS 84), elevation 2,918 m (Fig. 1). Ten of these salamanders were found under bunch grass, another six were found ca. 30 m away under a dry *Yuca*, seven more were found ca. 15 m away under another dry *Yuca*, and two additional salamanders were found under a rock.

Morphological description. Among the 26 captured *I. naucampatepetl*, SVL spanned 23–83.2 mm (mean \pm SD: 77.86 ± 10.75), TL varied from 0–73.4 mm (67.47 ± 10.18), T ranged from 23–156.6 mm (145.33 ± 20.93), and weight varied from 0.1–23 g (10.86 ± 2.83 ; Table 1). One individual with no tail had the smallest SVL value. Four of the 26 animals (with SVL ranging from 62.4–85 mm; Table 1) had clearly visible rounded mentonian glands and prominent nasolabial protuberances, suggesting they were sexually mature males. Another six animals were greater than 62.4 mm in SVL (range: 69.5–83.2 mm; Table 1), but lacked both mentonian glands and prominent nasolabial protuberances, so we concluded that they were adult females. The remaining salamanders were smaller than the smallest sexually mature male, so we considered them to be juveniles of undetermined sex.

Color pattern variation. All 26 captured *I. naucampatepetl* display a solid black body color with pale marks; these marks are orange in smaller individuals and pink or pinkish cream in larger individuals (Fig. 2). In all animals, the pale marks are arranged in a consistent pattern as follows: a pair of spots on the back of the head that vary in size, but are usually about the diameter of the orbit; a pair of inverted, rather elongated triangular marks on the shoulders; one to 11 pairs of small spots positioned dorsolaterally on the intercostal areas of the trunk; and a large, conspicuous mark on the caudosacral region that resembles a pelvis bone, ranging from a rounded to more quadrangular U-shape, and always with two small, rounded black marks. In addition, some individuals show one or two rounded orange, pink, or pinkish-cream spots in the nuchal region. The venter is pale to dark gray, and the mentonian gland in adult males is pale gray (Fig. 2).

Literature records. The work of Pérez y Soto (2016) reported some records and a photograph of a salamander found in Tetela de Ocampo, Puebla, Mexico, which was misidentified as *I. gigantea* but actually corresponds to *I. naucampatepetl*. Two of those specimens were deposited in the Herpetological Collection of the Zoological Museum Alfonso L. Herrera (MZFC 28819–20). That

Table 1. Morphological measurements of *Isthmura naucampatepetl* from the Municipality of Chignautla, Puebla, Mexico. Snout-vent length = SVL, tail length = TL, total length = T, all measurements in mm. Note: Specimen numbers match the numbers of photographs in Fig. 1.

Specimen number	Sex	SVL (mm)	TL (mm)	T (mm)	Weight (g)
1	Female	83.8	69	152.8	3
2	Female	83.2	73.4	156.6	23
3	Female	78.2	73	151.2	14
4	Female	78.1	69.8	147.9	11
5	Male	66.9	60.1	127	9
6	Male	85	70.7	155.7	10
7	Female	69.8	56.3	126.1	6
8	-	52.3	46.7	99	5
9	-	59.7	45.3	105	5
10	-	54.4	33.5	87.9	4
11	-	48.6	34.7	83.3	3
12	-	59.7	45	104.7	4
13	Female	69.5	56.7	126.2	9
14	-	47.9	26	73.9	4
15	-	60.9	42.5	103.4	6
16	Male	63.7	44.9	108.6	6
17	-	34	22.3	56.3	1.5
18	Female	73.8	54.7	128.5	5
19	Male	62.4	43.2	105.6	3
20	-	38.2	23.2	61.4	1
21	-	37.3	21.5	58.8	0.5
22	-	40.5	21.4	61.9	1
23	-	22.3	11	33.3	0.2
24	-	23	0	23	0.1
25	-	61.9	46.7	108.6	4.5
26	-	56.1	38.8	94.9	3
Mean		77.86	67.47	145.33	10.86
SD		10.75	10.18	20.93	2.83

author did not include the precise number of the observed individuals, but she described it as a “common species,” a category assigned in that work to the species with an abundance of 12–22 individuals.

Naturalista records. As mentioned above, this review includes the two records for *I. naucampatepetl* that were uploaded into the portal Naturalista, CONABIO (<https://www.naturalista.mx/search?q=Isthmura%20naucampatepetl>; Accessed: 1 September 2019). These photographs were taken on 6 October 2015 in the Municipality of Teziutlán, near the localities of San Juan Acateno and Atoluca, and they were published in Naturalista without precise locality information (Fig. 1, plus signs).

Habitat description. The site where the new population of *I. naucampatepetl* was found is (currently) under forest

management, which includes various activities such as fire protection, reforestation, soil conservation and restoration, and wood harvesting. All the forest management in the area is performed according to the standard methodologies in México as specified in *Método Mexicano de Ordenación de Bosques Irregulares* and the *Método de Desarrollo Silvícola*. In the specific site of specimen collection, the landscape is dominated by trees of the genera *Pinus*, *Quercus*, *Abies*, and *Alnus*, that vary in size and age, within ranges of 1.5–38 m high and 1–60 years old.

Discussion

Isthmura naucampatepetl was recently rediscovered in the state of Puebla, with two photographs uploaded in the Naturalista portal and cited by Aguilar-López et al. (2019). The work reported here confirms the presence of the species in another population in Puebla for the first time (García-



Fig. 2. Color patterns of each captured *Isthmura naucampatepetl* individual. Photo numbers correspond to the specimen numbers given in Table 1. Photo 27 is a ventral view of the chins of an adult female (left) and an adult male (right), showing the male mentonian gland. Photos by L. Fernández-Badillo.

Vázquez et al. 2009; Woolrich-Piña et al. 2017) and records the highest number of individuals reported for any known population. These records extend the geographic distribution of the species by 75.43 km from the report of Pérez y Soto (2016), 38 km from the reports of Naturalista, and 36.9 km (field records) from the type locality (Parra-Olea and Wake 2001; Parra-Olea et al. 2005). These records show this species has a wider distribution in the Sierra Norte of Puebla than was previously known, so additional search efforts in other regions of this area, with similar environmental and microhabitat conditions, could potentially reveal other populations and provide valuable data on the natural history of this rarely-encountered species.

During the field work for this study, many individuals of *I. naucampatepetl* were found grouped together, but we are currently unable to explain the reason for this behavior. However, local residents mentioned that in the previous year they unearthed a congregation of ca. 50 individuals

of the species while extracting soil near the area where our *I. naucampatepetl* were encountered, suggesting that this behavior may be a regular occurrence.

The color pattern of the 26 individuals found generally agrees with the description available in Parra-Olea and Wake (2001), but some variations are described above and clearly evident in Fig. 2. The data from this population modestly increase the maximum known SVL of adult females from 82.9 mm (Parra-Olea and Wake 2001) to 83.2 mm. For males, Parra-Olea and Wake (2001) reported that sexually mature individuals vary from 67.6–82.1 mm SVL, a range that our data broadens (62.4–85 mm SVL).

This contribution increases our knowledge of the species regarding its conservation. The IUCN (2020) indicates a decreasing current population trend for *I. naucampatepetl*. In contrast, we recorded a strong signal of demographic variability in the newly discovered population, including two individuals of less than 50 mm T, nine of 56.3–99

mm T, and 15 of 103.4–156.6 mm T. This size variation distribution suggests that the population is actively reproducing, but more observations and long-term study are necessary to rigorously assess the population size and trends in this species.

The IUCN (2016) identifies extensive logging, farming (especially for potatoes), and expanding human settlements as the major threats to *I. naucampatepetl* at the type locality in Veracruz. At the new locality (Bienes Comunales San Mateo Chignautla, Puebla), the land owners have a legal permit to sell wood from the forest, but we also detected some other disturbances (like cattle, feral and domestic dogs, some threats inherent to the forest management, and also wildfires) which can pose risks for this species.

Adequate protection of this species would benefit from the inclusion of management actions specifically related to *I. naucampatepetl* in an updated forest management plan, and perhaps the establishment of conservation areas where timber extraction can be limited. To better inform decisions on how to preserve this newly discovered population, there is an urgent need to acquire basic natural history and population data. It is also imperative to educate the local inhabitants about the global importance of this salamander and the need to conserve it. Grassroots buy-in is necessary for any successful conservation strategy, as is involvement by governmental and non-governmental stakeholders. If coordinated in alliance with local residents and landowners, and linked with forest management strategies, we can show that the conservation of *I. naucampatepetl* and the sustainable use of the forest go hand in hand.

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Literature Cited

- Aguilar-López JL, García-Buñuelos P, Pineda E, Rovito SM. 2019. *Thorius narismagnus* (Amphibia: Plethodontidae): rediscovery at the type locality and detection of a new population. *Amphibian & Reptile Conservation* 13(2) [General Section]: 126–132 (e193).
- Bishop PJ, Angulo A, Lewis JP, Morre RD, Rabb GB, Garcia-Moreno J. 2012. The amphibian extinction crisis: what will it take to put the action into the amphibian conservation action plan? *S.A.P.I.E.N.S* 5: 97–111.
- Furbush R, Caviedes-Solis IW, Méndez de la Cruz FR, Leache AD. 2017. Missing in action for nearly 50 years: rediscovery of the Mexican treefrog *Duellmanohyla ignicolor* (Anura: Hylidae). *Mesoamerican Herpetology* 4: 120–127.
- García-Bañuelos P, Aguilar-López JL, Pineda E, García-Vinalay A. 2017. Rediscovery of *Bromeliohyala dendroscata* at the type locality: a threatened treefrog surviving in a highly human modified landscape in Mexico. *Mesoamerican Herpetology* 4: 684–688.
- García-Vázquez UO, Canseco-Márquez L, Gutiérrez-Mayén G, Trujano-Ortega M. 2009. Actualización del conocimiento de la fauna herpetológica en el estado de Puebla, México. *Boletín de la Sociedad Herpetológica Mexicana* 17: 12–36.
- IUCN SSC Amphibian Specialist Group. 2016. *Isthmura naucampatepetl*. The IUCN Red List of Threatened Species 2016: e.T59389A53983432.
- IUCN. 2018. The IUCN Red List of Threatened Species Version 2017-3. Available: <http://www.iucnredlist.org> [Accessed: 3 December 2019].
- Naturalista (CONABIO). 2020. *Isthmura naucampatepetl*. Available: <http://www.naturalista.mx/taxa/2488107-Isthmura-naucampatepetl> [Accessed: 12 February 2020].
- Parra-Olea G, Wake DB. 2001. New species of lungless salamanders of the genus *Pseudoeurycea* (Amphibia: Caudata: Plethodontidae) from Veracruz. *Scientific Papers, Natural History Museum, University of Kansas* 20: 1–9.
- Parra-Olea G, García-París M, Papenfus TJ, Wake DB. 2005. Systematics of the *Pseudoeurycea belli* (Caudata: Plethodontidae) species complex. *Herpetologica* 61: 145–158.
- Pérez y Soto R. 2016. Anfibios y reptiles de Tetela de Ocampo, Puebla. B.Sc. Thesis. Benemérita Universidad Autónoma de Puebla, Puebla, Mexico.
- WFC (Wild Forest Consulting), ARST (Asociación Regional de Silvicultores de Teziutlán). 2018. *Estudio Regional de Fauna Silvestre en Bosques de Producción en la UMAFOR 2103 Teziutlán, Puebla*. Wild Forest Consulting S.C. and Asociación Regional de Silvicultores de Teziutlán A.C. Comisión Nacional Forestal, Puebla, México. 257 p.
- Woolrich-Piña GA, García-Padilla E, DeSantis DL, Johnson JD, Mata-Silva V, Wilson LD. 2017. The herpetofauna of Puebla, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 790–884.
- ZSL (Zoological Society of London). 2008. EDGE of Existence Programme. Available: <http://www.edgeofexistence.org/index.php> [Accessed: 10 August 2020].



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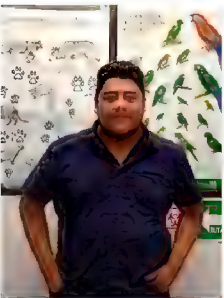
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Unearthing the species diversity of a cryptozoic snake, *Tantilla melanocephala*, in its northern distribution with emphasis on the colonization of the Lesser Antilles

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Abstract.—*Tantilla* is a diverse New World Colubrid genus comprising 69 small to medium sized, cryptozoic and semi-fossorial species. Morphological data of *Tantilla melanocephala* in the Eastern Caribbean region, and more precisely on the islands of Trinidad and Tobago and nearby Venezuela, have shown differences in scales and color patterns associated with these localities, which may suggest the presence of cryptic species in the region. Assessing the monophyly of *Tantilla melanocephala* is key as its paraphyly could compromise important decisions for conservation and management. In this study, we conduct phylogenetic analyses of all available *Tantilla* from GenBank ($n = 11$), including *T. melanocephala* from French Guiana and Brazil, along with novel sequences from Guyana, Venezuela, Trinidad, and Tobago. Broadly, we recover two sister clades within *Tantilla*, a North American-Central American clade and a Central American-South American clade with a time since its most recent ancestor dating to the Mid-Miocene. We found the sampled *T. melanocephala* to be monophyletic in all analyses and estimated the origin of this clade towards the early Pleistocene. The close association between Trinidad and Venezuela, dating towards the end of the Pleistocene, suggests connections in the recent past. This study is the first to assess the intraspecific variation in the species and we hope it will set a landmark to complete the systematic characterization of the entire species throughout its widespread distribution.

Keywords. Biogeography, colonization, dispersal, Reptilia, Tobago, Trinidad

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Introduction

Despite the widespread distribution ranges of certain cryptic species, their presence on islands often reveals lineages divergent from those on the mainland and even the presence of new species (Card et al. 2016; Jowers et al. 2019; Murphy et al. 2019a,b). Therefore, an understanding of both the ecology and evolutionary history of an endangered species on islands are pivotal for ensuring their effective protection and conservation (Young 2000; Spielman et al. 2004). The Trinidad and Tobago Archipelago is located on the continental shelf

of northern South America with the Caribbean Sea to the north, the Atlantic Ocean to the east, and the Gulf of Paria to the west. It is composed of two main islands (Trinidad and Tobago) and about 20 smaller satellites and offshore rocks. While both larger islands are considered the southernmost Lesser Antilles, they have a continental flora and fauna with two distinctly different geological origins. Trinidad was previously attached to Venezuela and formed by a pull-apart basin in the Late Miocene, when a downward warping event separated both land masses (Liddle 1946; Erlich and Barrett 1990). Tobago, on the other hand, was formed as an oceanic island on

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the front edge of the Caribbean Plate far to the West of its current position (Pindell and Kennan 2007; Jowers et al. 2015). These islands can be considered as an eastward extension of the sky island complex formed by the Venezuelan coastal ranges. Their geographic position is also unique because they lie just to the north of the Guiana Shield at the mouth of the Orinoco River (Fig. 1).

Widespread taxa that span across biogeographic barriers can pose a particular challenge for taxonomists as isolation processes can often lead to the presence of cryptic lineages. Barriers to gene flow allow opportunities for speciation, and their removal offers the opportunity for secondary contact that may result in introgression. Species that are widely distributed are also dispersing from multiple distant locations, and genetic material from these populations could converge at more proximate geographic localities with the assistance of wind or water (Reynolds et al. 2020). Furthermore, these species are key for understanding how diverse ecological factors may drive regional patterns of species divergence and speciation (Card et al. 2016). Fortunately, advances in molecular phylogenetics can provide resolution to our understanding of the evolutionary history of even rarely studied species.

The black-headed snakes of the genus *Tantilla* are small to medium sized (usually < 300 mm) Western Hemisphere snakes that specialize in feeding on arthropods, particularly centipedes (Marques and Puerto 1998). Currently, the genus comprises 69 species (Uetz et al. 2020) distributed from sea level to at least 3,000 m and ranges from Nebraska (USA) to Santa Fe Province (Argentina). It is present on both the Pacific and Atlantic versants from Guatemala southward through Central America into South America, reaching as far south as southern Peru, Bolivia, northern Argentina, and Uruguay; and it is also present in the Lesser Antilles, on the Trinidad and Tobago islands (Henderson and Powell 2006, 2018). *Coluber melanocephala* Linnaeus (1758) was the first member of the genus described, and it has

been re-described at least ten times since Linné's original description (Wilson and Mena 1980). The distribution of *T. melanocephala* as currently understood covers much of the Neotropics, from Colombia to northern Argentina and Uruguay, including the islands of Trinidad and Tobago (Fig. 1). Recently, this species was recorded on St. Vincent and Grenada banks, where its presence has been documented since at least 2005, and it presumably arrived through human-mediated introduction in construction material from Guyana and probably also from Trinidad and Tobago (De Silva and Wilson 2006; Henderson and Powell 2006, 2018; Berg et al. 2009; Henderson and Breuil 2012). Records from Panama correspond to misidentifications of *T. armillata* (Ray 2017). Like some congeners, it is also present on both sides of the Andes. Greenbaum et al. (2004) synonymized *T. equatoriana* Wilson and Mena 1980 with *T. melanocephala* based on a morphometric data principal component analysis.

The phylogenetic position of *Tantilla* remains inconclusive as several molecular phylogenetic studies have found alternative placements of the genus among the Colubridae (Pyrón et al. 2013; Jadin et al. 2014; Koch and Venegas 2016; Figueroa et al. 2016; Zaher et al. 2019). Pyron et al. (2013) used only *T. melanocephala* as a representative for the genus in their squamate study, finding *Tantilla* and *Salvadora mexicana* to be the sister to *Coluber* and *Masticophis*. This generic placement of *Tantilla* contrasted with Jadin et al. (2014) who found *T. relictus* as sister to *Conopsis*, *Pseudoficimia*, and *Sympholis*. However, both of these relationships were later confirmed by Figueroa et al. (2016) who included more *Tantilla* taxa and found the genus to be paraphyletic, reached similar conclusions to Pyron et al. (2013) and Jadin et al. (2014), and consequently considered its placement in the larger snake phylogeny unresolved. Koch and Venegas (2016) included *T. amilatae*, *T. impensa*, *T. melanocephala*, and *T. vermiformis* in their description of *T. tjiasmantoi* but had no support for intergeneric

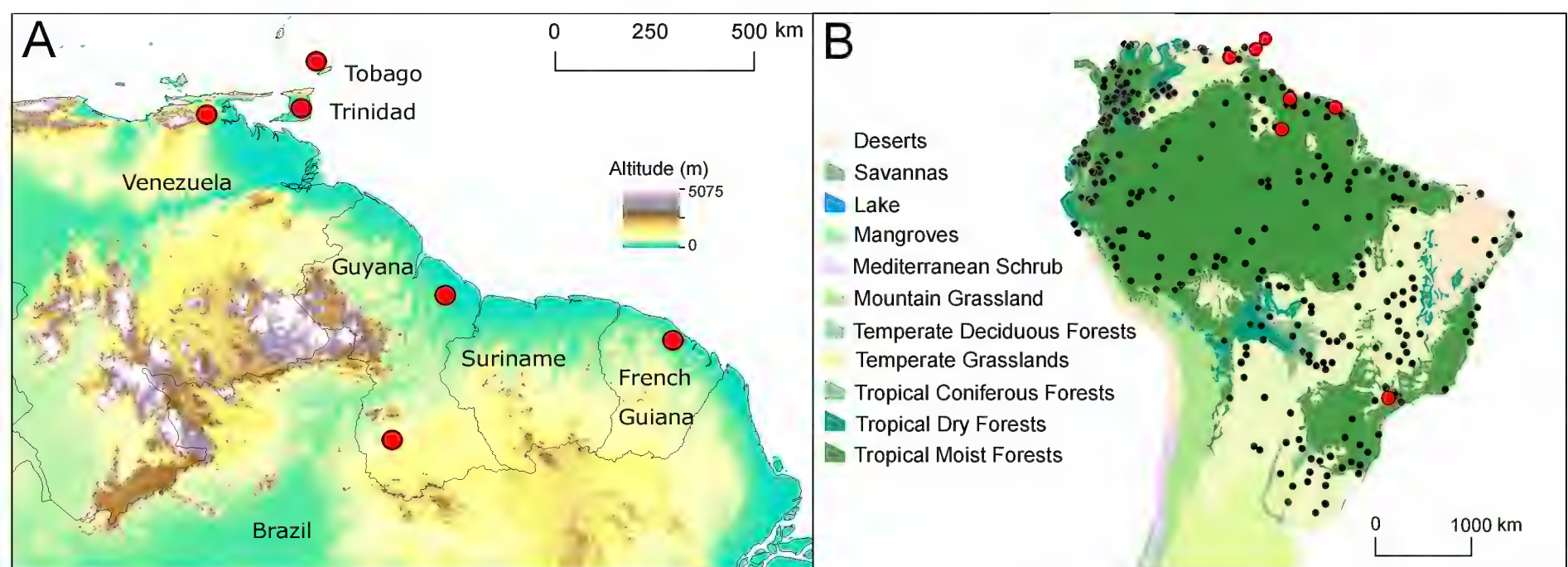


Fig. 1. (A) *Tantilla melanocephala* sample localities for this study (red circles) in the northern region of its distribution. (B) The distribution of *Tantilla melanocephala* in the Neotropics. Locality data are from VertNet and the GBIF databases, as well as the literature (Nogueira et al. 2019). Within the Lesser Antilles, Union Island and the Mustique islands are not shown. Red circles are *T. melanocephala* localities included in the phylogenetic analyses. The map suggests this species inhabits several different biomes.

relationships. Zaher et al. (2019) reported *T. relicta* and *T. melanocephala* as being sister to *Scolecophis*, and together they were sister to a clade of colubrids consisting of *Conopsis*, *Ficimia*, *Gyalopion*, *Pseudoficimia*, *Sympholis*, *Sonora*, and *Stenorrhina*.

Along with *Boa constrictor*, *Tantilla melanocephala* is among the most widely distributed snakes in South America. *Tantilla melanocephala* is distinguished from congeners by the following combination of traits: (1) dark head cap transitions into dark nape band with two pale spots covering the posterior parietals, posterior edge of temporals, and associated post parietal scales; (2) pale preocular spot; (3) lateral extension of the head cap contacts gulars; (4) no pale nape band posterior to the dark nape band; (5) background color tan with nine dark brown stripes; and (6) a greater number of subcaudals compared to other members of the *T. melanocephala* group. *Tantilla melanocephala* is characterized by extensive geographic variation in the color pattern, a wide range of ventral (126–177 ventrals) and subcaudal counts (41–74), as well as occupation of seven different biomes (Wilson and Mena 1980; Wilson 1992). Several attempts to clarify the marginal populations have been conducted superficially based in their morphology, mainly on color patterns and cephalic scutellation (Wilson and Mena 1980; Vuoto 1998). Wilson and Mena (1980) discussed the variation of *T. melanocephala* ventral scale counts in two Caribbean islands, noting that the differences in ventral counts between specimens from Trinidad, adjacent Venezuela, and Tobago, are striking (Fig. 2). Similarly, the authors found significant differences in subcaudals between mainland Venezuela and the islands. In addition, six color patterns were detected across the range in *T. melanocephala* by Wilson and Mena (1980), with Trinidad and Tobago specimens expressing two of these patterns along with specimens from Argentina, Colombia, Guyana, Paraguay, Suriname, Uruguay, and Venezuela.

Herein, we assess whether populations from the islands of Trinidad and Tobago, and proximal mainland localities of Venezuela (Peninsula de Paria) and Guyana, constitute the same lineage or if they are part of a cryptic lineage complex. Furthermore, molecular phylogenetics are used to examine evolutionary relationships within *Tantilla*. Finally, the likely time of colonization from the mainland is explained, along with colonization of the islands in relation to climatic conditions in the region. Given the morphological variation between the two island populations that are now separated by 50 km of water and were once connected to each other at glacial maxima when sea levels had dropped, the genetic distance between the two populations is investigated.

Materials and Methods

Tantilla melanocephala specimens were collected from locations in Trinidad, Tobago, and Venezuela, under licenses from the Trinidad and Tobago Government



Fig. 2. Specimens of *Tantilla melanocephala* from (A) Tobago, Pigeon Point, (B) Trinidad, Bush Bush, Nariva Swamp, and (C) Venezuela, Caracas, Distrito Capital. Photos by J.C. Murphy (A–B) and L.A. Rodríguez (C).

Wildlife Section: Special Game Licenses issued for scientific purposes in 2015–2016 to John Murphy, Renoir Auguste, and Mike Rutherford; and under collection permit number 1,375 granted to Gilson A. Rivas by the Ministerio del Poder Popular para Ecosocialismo y Aguas, Venezuela. Animals were euthanized following the ASIH guidelines (Beaupre et al. 2004) using pentobarbital. Museum acronyms follow Sabaj (2019).

DNA was extracted using a Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer (see Supplemental Table S1 for list of primers). The target genes were the mitochondrial small and large ribosomal subunits (12S

rDNA and 16S rDNA, respectively), cytochrome *b* (cytb) and the nuclear oocyte maturation factor (c-mos; see Supplemental Table S2). Sequence editing follows Murphy et al. (2019c). Despite some individuals having different lengths in some alignments, the lengths of the alignments were: 12S rDNA, 404 base pairs (bp); 16S rDNA, 494 bp; cytb, 1,086 bp; and c-mos, 561 bp. Cytb and c-mos were translated into amino acids to check for the presence of stop codons. Following Jadin et al. (2014, 2019), Figueroa et al. (2016), and Zaher et al. (2019), all genera that were sister to *Tantilla* were included, while *Drymarchon couperi* and *D. corais* were used as outgroup (Supplemental Table S2). Sequences were aligned in Seaview v4.2.11 (Gouy et al. 2010) under ClustalW2 default settings (Larkin et al. 2007) for the cytb and c-mos fractions, and with MAFFT (Kato et al. 2002) for the 12S and 16S rDNA. The 12S and 16S rDNA, cytb, and c-mos sequences were concatenated, resulting in a 2,548 bp alignment.

Phylogenetic analyses were performed using the Bayesian Inference and Maximum Likelihood methods. MrBayes v3.2 (Ronquist and Huelsenbeck 2003) was used to construct the Bayesian Inference tree under the best-fitting substitution model for each gene partition. ML searches were conducted in RAxML v7.0.4 (Silvestro and Michalak 2012), using partition data sets under default settings, and support was assessed by using 1,000 bootstrapped replicates. The most appropriate substitution model was implemented for each gene fragment as determined by the Bayesian Information Criterion in PartitionFinder v2 (Lanfear et al. 2017) to choose the optimal partitioning strategy for both phylogenetic analyses (Supplemental Table S3). Default priors and Markov chain settings were used, and searches were performed with random starting trees. Each run consisted of four chains of 20 million generations, sampled every 2,000 generations.

BEAST v1.8.4 (Drummond et al. 2012) was used to simultaneously estimate the phylogeny and divergence times between taxa. The most appropriate substitution model was implemented for each gene fragment as determined by the Bayesian Information Criterion in jModeltest v2 (Posada 2008). A Yule speciation tree prior was applied, along with a relaxed lognormal clock for the concatenated 12S+16S rDNA and for the c-mos alignments. A strict clock, using a substitution rate of 1.34% substitutions per million years, was applied for the cytb gene fragments, as estimated by Daza et al. (2009) for Neotropical colubrids based on four calibration points. As priors for the rates, the approximate reference (CTMC) prior was selected (Ferreira and Suchard 2008). BEAST was run twice with 50 million generations per run, sampling every 5,000 steps. Convergence of the runs was verified in Tracer v1.6 (Rambaut et al. 2013), both runs were combined in LogCombiner, and the Maximum Clade Credibility Tree was computed using Tree Annotator (BEAST v1.8.4). All analyses were performed

through the CIPRES platform (Miller et al. 2010).

Results

No stop codons were found in the cytb and c-mos alignments. The best-fitting models are shown in Supplemental Table S3. All phylogenetic analyses recovered *Tantilla melanocephala* as monophyletic (Figs. 3–4). Similarly, all analyses recovered a strongly supported *T. melanocephala* from Venezuela+Trinidad clade. The relationship with Tobago is weakly supported (Fig. 3). The timing of the most recent common ancestor of *Tantilla* dates to the Middle Miocene (~12 Mya: 95% HPD 10.7–14.3 Mya), but this time estimate is likely to change when other species are included in future phylogenetic analyses (Fig. 4). Timing of the *T. melanocephala* clade varies considerably, with the time since the most recent common ancestor (TMRCA) dating to the beginning of the Pleistocene (2.3 Mya: 95% HPD 1.8–3 Mya). This early split relates to the timing between *T. melanocephala* from Brazil and all other localities of *T. melanocephala*. The divergence between Guyana + French Guiana and Venezuela + Trinidad + Tobago dates to 1.8 Mya (95% HPD 1.2–2.4 Mya) (Fig. 4). A more recent TMRCA towards the end of the Pleistocene is recovered between Trinidad and Venezuela *T. melanocephala* (0.2 Mya, 95% HPD 0.014–0.48 Mya). The recovered sister clade relationship between *T. tjiasmantoi* (from Peru) and *T. melanocephala* requires further investigation as *T. tjiasmantoi* is the only *Tantilla* species missing cytb and c-mos sequence data.

Discussion

The analyses presented here recovered two clades within *Tantilla*, a North American-Central American clade consisting of *T. coronata*, *T. gracilis*, *T. hobartsmithi*, *T. impensa*, *T. nigriceps*, *T. planiceps*, and *T. relictata* and a Central American-South American clade with *T. armillata*, *T. vermiformis*, *T. tjiasmantoi*, and *T. melanocephala*. The calibration estimates for *T. melanocephala* divergence reject a vicariant event between Trinidad and northern South America in the Late Miocene when Trinidad detached from the Peninsula de Paria of northern Venezuela ~4 Mya (Babb and Mann 1999; Flinch et al. 1999), and point to a divergence in the Late Pleistocene. The Late Pleistocene was a time of rapid and abrupt topographic change in the eastern Caribbean associated with eustatic sea level changes in the region (Murphy 1997; Hansen et al. 2013; Murphy et al. 2019c). The low genetic divergence of *T. melanocephala* recovered between Venezuela and Trinidad is similar to that found in *Atractus trilineatus* between Guyana and the islands (Murphy et al. 2019c), and it likely results from the changing topographical conditions that facilitated the connections between regions (Murphy 1997; Murphy et al. 2019c). Different parts of the Trinidad and Tobago

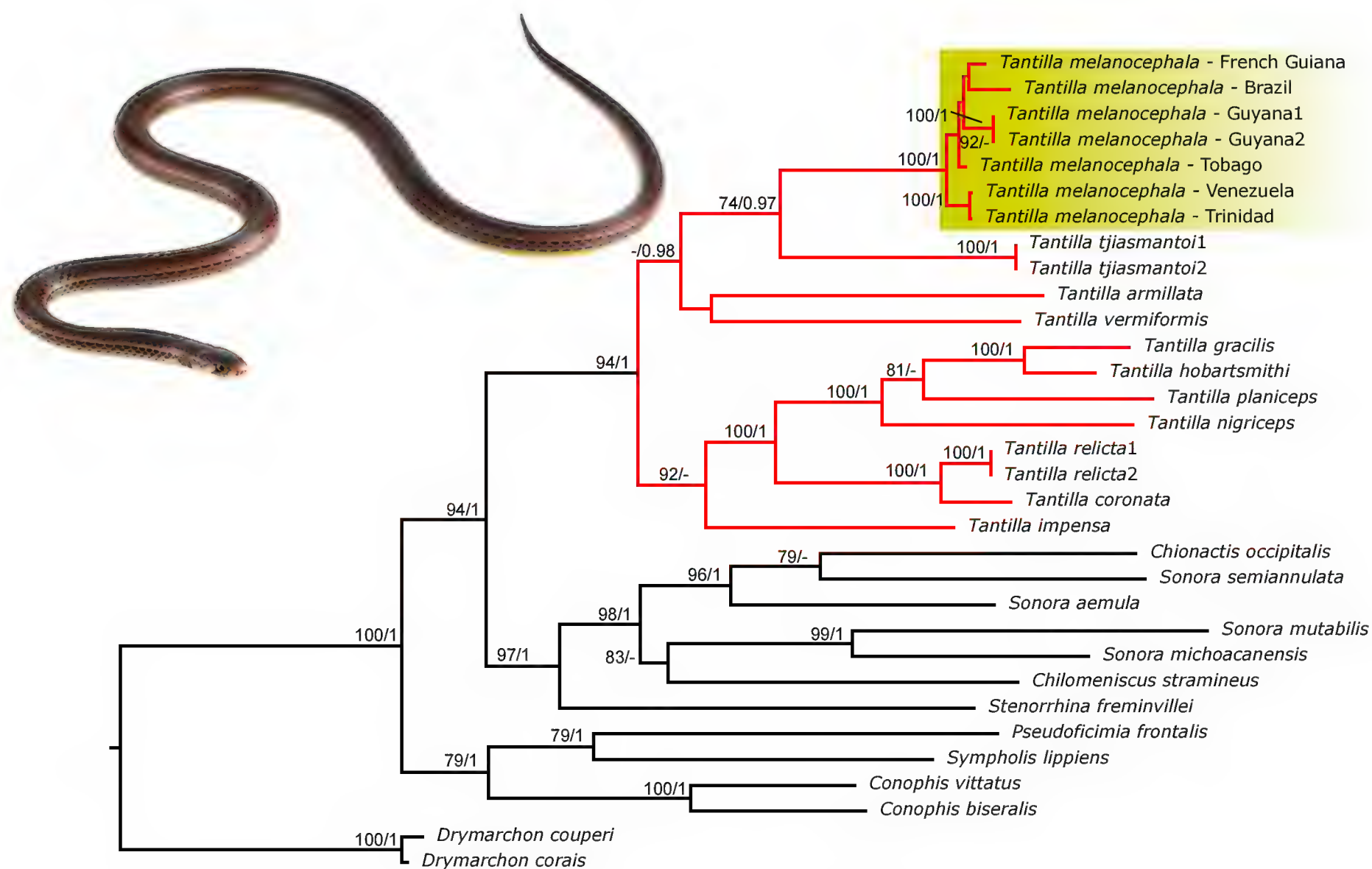


Fig. 3. Best Maximum Likelihood tree based on the data set of concatenated 12S and 16S rDNA, cytb, and c-mos sequences. Red clade depicts the genus *Tantilla*. Values on the left and right sides of a slash (/), are the values indicated at nodes of Maximum Likelihood bootstraps (>70%) and Bayesian Posterior probability values (>95%), respectively. The *Tantilla melanocephala* pictured is from the western versant of the Occidental slopes in Ecuador (from the Rio Manduriacu Reserve). *Photo by R. Maynard.*

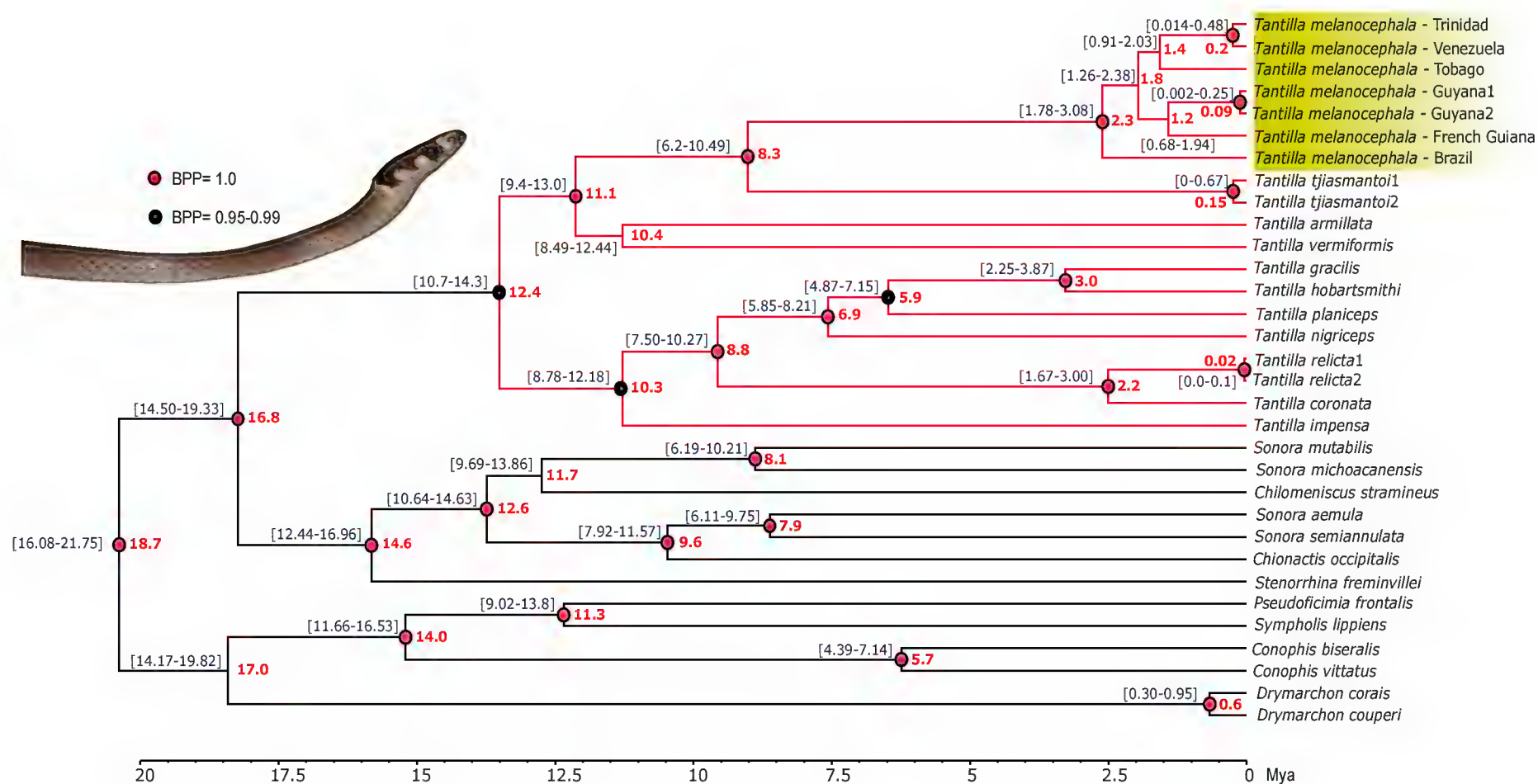


Fig. 4. Bayesian time tree as inferred by BEAST for the data set of concatenated 12S and 16S rDNA, cytb, and c-mos sequences from *Tantilla* specimens (in red). Red values by nodes denote the median time estimates, whereas values in brackets denote 95% Highest Posterior Density ranges. Red and black nodes are posterior probabilities (1.00 and > 95–99%), respectively. *Photo by J.C. Murphy.*

archipelago were connected to the mainland multiple times, with Trinidad connected to the mainland for more prolonged periods than Tobago (Murphy et al. 2019c).

When sea levels dropped during the Pleistocene, gene flow could be expected between island populations and the mainland, as connections could be established with sea level drops as moderate as 50 m (Murphy 1997; Hansen et al. 2013). Thus, recent divergences in a variety of organisms were likely. For example, Robinson's Mouse Opossum (*Marmosa robinsoni*) from Venezuela and Trinidad and Tobago has been shown to diverge 0.34 Mya (Gutierrez et al. 2014), and the skinks *Marisora aurulae* from Trinidad and *M. falconensis* from Estado Falcón in the Paraguaná Peninsula, diverged ca. 0.5 Mya (Hedges and Conn 2012). Therefore, vicariance is more plausible than an over-water dispersal during the interglacial periods. Nevertheless, over-water dispersal cannot be ruled out as an explanation for the arrival of species in Tobago from Trinidad (e.g., Boos 1984a,b; Camargo et al. 2009; Murphy et al. 2016). For example, the shared *Micrurus diutius* haplotypes in Trinidad and Guyana and the low divergence of *Atractus trilineatus* from Trinidad, Tobago, and Guyana suggest dispersal through either Pleistocene land bridge formations or rafting (Murphy et al. 2019c).

Furthermore, recent work on the fossorial Trinidad Threadsnake, *Epictia tenella*, has shown a remarkable genetic similarity between Guyana and Trinidad, suggesting colonization by recent dispersal rather than ancient vicariance (Murphy et al. 2016). The results of the current study indicate that mainland southern localities (Guyana and French Guiana) show deeper divergence compared to Venezuela-Trinidad and Tobago, with a TMRCA dating to circa 1.8 Mya and a basal split between Brazil and the rest of the clade dating to 2.3 Mya. Similarly, the frog *Leptodactylus validus* originates from northern South America (Guyana/Brazil) and its dispersal to Trinidad ~1 Mya has been attributed to periods of intermittent land connections, or overwater dispersal, throughout the Pleistocene (Camargo et al. 2009). This pattern was also reported for *Atractus trilineatus* between individuals from coastal Guyana and Trinidad-Tobago (Murphy et al. 2019c). Indeed, dispersals from the northern mainland to Trinidad may be a regular occurrence and a source of close genetic associations with local herpetofaunal populations (Charles 2013). More effective conservation measures can thus be implemented based on increased knowledge of the distribution and systematics of relevant species (Bohm et al. 2013).

The recovery of a strongly monophyletic clade contrasts with what we might have expected based on morphological data (Wilson and Mena 1980). Combining data from Wilson and Mena (1980) and the data presented here, the mean ventral counts on Trinidad males (146.8) are lower by approximately 5.8 scales than on animals from adjacent Venezuela (where males average 152.6);

for females the difference is even greater at 9.3 scales (Trinidad averages 150.7 ventrals and adjacent Venezuela averages 160). Comparing Tobago and Trinidad, male ventral numbers again increase markedly in Tobago over those in Trinidad, and are greater than those from mainland Venezuela. Wilson and Mena (1980) also commented that only specimens from upper Central America have higher ventral counts than those from Tobago. Comparable figures for Tobago females were not available, but the count for the single Tobago female (168) was nine scales more than the highest count for Trinidad females. In contrast, subcaudal counts were higher in Venezuela (male average 66.4) and Trinidad (male average 57.2), but subcaudal counts in Tobago males averaged 73.25. Additional population sampling will be required from marginal populations (such as those in Argentina, Uruguay, southern Brazil, and west of the Andes) to encompass the wider distribution of the species and ascertain the complete range of morphological variation in the species.

Our phylogenetic analyses provide evidence for the idea that at least a portion of the Trinidad and Tobago herpetofauna has closer ties to the Caribbean Coastal Ranges (CCR) of Venezuela than to the Guiana Shield. Distribution patterns and molecular evidence from other taxa, such as various frogs (*Flectonotus fitzgeraldi*, *Hyalinobatrachium orientale*, *Mannophryne olmonae*, *M. trinitatis*, *Pristimantis charlottevillensis*, *P. turpinorum*); lizards (*Anolis* cf. *tigrinus*, *Bachia trinitatis*, *Gonatodes ceciliae*, *G. ocellatus*, *Oreosaurus shrevei*, *Plica caribea*, *Polychrus auduboni*); and snakes (*Atractus fuliginosus*, *Dipsas variegata*, *Erythrolamprus bizona*, *E. pseudoreginae*, *Ninia atrata*, *Micrurus circinalis*), corroborate a shared fauna between Trinidad, Tobago, and the CCR (Murphy 1997; Angarita-Sierra 2014; Jowers et al. 2015; Murphy et al. 2018). However, *Tantilla melanocephala* is a widespread species that also has a shared genetic history with the CCR despite its proximity to the Guiana Shield. We suspect that as more species are examined this pattern will become more prevalent.

Conservation

The total number of *Tantilla melanocephala* individuals observed on Trinidad and Tobago over 15 field trips was only 12 (JCM field notes). In contrast, Lynch (2015) found *T. melanocephala* to be among the five most frequently encountered snakes in African Oil Palm plantations that were sampled in the department of Meta, Colombia. However, *T. melanocephala* was among the rarest snakes found in oil palm plantations at all other sites sampled across Colombia. Such findings suggest that *T. melanocephala* might be associated with certain habitat types or dynamics (e.g., oil palm plantations surrounded by forest as opposed to pasture), being very common in some areas or very rare in others. However, it

is also plausible that the *T. melanocephala* data reported by Lynch (2015) are representative of more than one lineage, suggesting that some populations are more tolerant of disturbed habitats than others.

Our study shows the lack of cryptic species diversity within a few regions of the peripheral populations of *T. melanocephala*. This finding implies that (at least for now) its conservation status of Least Concern (IUCN 2020) is suitable. Increasing the collection and sequencing efforts across most of the distribution range of this species will be challenging, but such an effort would likely address the presence of population diversity and morphotypes from different habitats. In particular, sampling of biogeographically important regions (e.g., Trans-Andean) might reveal divergent lineages that warrant a more protective conservation status.

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Literature Cited

- Angarita-Sierra T. 2014. Hemipenial morphology in the semifossorial snakes of the genus *Ninia* and a new species from Trinidad, West Indies (Serpentes: Dipsadidae). *South American Journal of Herpetology* 9: 114–130.
- Babb S, Mann P. 1999. Structural and sedimentary development of a Neogene transpressional plate boundary between the Caribbean and South American plates in Trinidad and the Gulf of Paria. Pp. 495–557 In: *Caribbean Sedimentary Basins. Elsevier Basins of the World*. Editor, Mann P. Elsevier, Amsterdam, Netherlands. 699 p.
- Beaupre SJ, Jacobson ER, Lillywhite HB, Zamudio K. 2004. *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research*. American Society of Ichthyologists and Herpetologists, Lawrence, Kansas, USA. 14 p.
- Berg CS, Jeremiah A, Harrison B, Henderson RW. 2009. New island records for *Tantilla melanocephala* (Squamata: Colubridae) on the Grenada Bank. *Applied Herpetology* 6: 403–404.
- Bohm M, Collen B, Baillie JEM, Zug G. 2013. The conservation status of the world's reptiles. *Biological Conservation* 157: 372–385.
- Boos HEA. 1984a. A consideration of the terrestrial reptile fauna on some offshore islands north west of Trinidad. *Living World, Journal of the Trinidad and Tobago Field Naturalists' Club* 1984: 19–26.
- Boos HEA. 1984b. Reptiles of Soldado Rock, Trinidad. *Living World, Journal of the Trinidad and Tobago Field Naturalists' Club* 1984: 12.
- Burbrink FT, Lawson R, Slowinski BB. 2000. Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* 54: 2,107–2,118.
- Camargo A, Heyer WR, de Sá RO. 2009. Phylogeography of the frog *Leptodactylus validus* (Amphibia: Anura): patterns and timing of colonization events in the Lesser Antilles. *Molecular Phylogenetics and Evolution* 53: 571–579.
- Card DC, Schield C, Shield DR, Richard AH, Corbin AB, Perry BW, Audra AL, Pasquesi GIM, Smith EN, Jezkova T, et al. 2016. Phylogeographic and population genetic analyses reveal multiple species of *Boa* and independent origins of insular dwarfism. *Molecular Phylogenetics and Evolution* 102: 104–116.
- Charles SC. 2013. An interesting reptile dispersal event from continental South America to Trinidad, Trinidad and Tobago. *Living World, Journal of The Trinidad and Tobago Field Naturalists' Club* 2013: 63.
- Daza JM, Smith EN, Páez VP, Parkinson CL. 2009. Complex evolution in the Neotropics: the origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae). *Molecular Phylogenetics and Evolution* 53: 653–667.
- De Silva M, Wilson D. 2006. *A Natural History of Mustique*. The Mustique Company, Ltd., Mustique Island, St. Vincent and the Grenadines. 331 p.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1,969–1,973.
- Erlich RN, Barrett SF. 1990. Cenozoic plate tectonic history of the northern Venezuela-Trinidad area. *Tectonics* 9: 161–184.
- Ferreira MA, Suchard MA. 2008. Bayesian analysis of elapsed times in continuous-time Markov chains. *Canadian Journal of Statistics* 36(3): 355–368.
- Figuroa A, McKelvy AD, Grismer LL, Bell CD, Lailvaux SP. 2016. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. *PLoS ONE* 11: e0161070.
- Flinch JF, Rambaran V, Ali W, De Lisa V, Hernandez G, Rodrigues K, Sams R. 1999. Structure of the Gulf of Paria pull-apart basin (Eastern Venezuela-Trinidad). Pp. 477–494 In: *Caribbean Sedimentary Basins. Elsevier Basins of the World*. Editor, Mann P. Elsevier, Amsterdam, Netherlands. 669 p.
- Goebel AM, Donnelly JM, Atz ME. 1999. PCR primers

- and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome *b* in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Molecular Phylogenetics and Evolution* 11: 163–199.
- Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4. A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27: 221–224.
- Greenbaum E, Carr JL, Almendáriz A. 2004. Taxonomic status of *Tantilla equatoriana* Wilson and Mena 1980 (Serpentes: Colubridae). *The Southwestern Naturalist* 49: 457–464.
- Gutierrez EE, Anderson RP, Voss RS, Ochoa-G J, Aguilera M, Jansa SA. 2014. Phylogeography of *Marmosa robinsoni*: insights into the biogeography of dry forests in northern South America. *Journal of Mammalogy* 95: 1,175–1,188.
- Hansen J, Sato M, Russell G, Kharecha P. 2013. Climate sensitivity, sea level, and atmospheric carbon dioxide. *Philosophical Transactions of the Royal Society A* 371: 20120294.
- Hedges SB, Conn CR. 2012. A new skink fauna from Caribbean islands (Squamata, Mabuyidae, Mabuyinae). *Zootaxa* 3288: 1–244.
- Henderson RW, Powell R. 2006. Geographic distribution, *Tantilla melanocephala*. *Herpetological Review* 37: 501.
- Henderson RW, Breuil M. 2012. Lesser Antilles. Pp. 150–161 In: *Island Lists of West Indian Amphibians and Reptiles*. Bulletin of the Florida Museum of Natural History, Volume 51, number 2. Editors, Powell R, Henderson RW. Florida Museum of Natural History, Gainesville, Florida, USA. [85]–166 p.
- Henderson RW, Powell R. 2018. *Amphibians and Reptiles of the St. Vincent and Grenada Banks, West Indies*. Edition Chimaira, Frankfurt am Main, Germany. 448 p.
- IUCN. 2020. Black-headed Snake, *Tantilla melanocephala*. The IUCN Red List of Threatened Species 2019: e.T203321A2763890.
- Jadin RC, Burbrink FT, Rivas GA, Vitt LJ, Barrio-Amorós CL, Guralnick RP. 2014. Finding arboreal snakes in an evolutionary tree: phylogenetic placement and systematic revision of the Neotropical birdsnakes. *Journal of Zoological Systematics and Evolutionary Research* 52: 257–264.
- Jadin RC, Blair C, Jowers MJ, Carmona A, Murphy JC. 2019. Hiding in the lianas of the tree of life: molecular phylogenetics and species delimitation reveal considerable cryptic diversity of New World Vine Snakes. *Molecular Phylogenetics and Evolution* 134: 61–65.
- Jowers MJ, Lehtinen RM, Downie RJ, Georgiadis AP, Murphy JC. 2015. Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): evidence for Pliocene connections between mainland Venezuela and the island of Tobago. *Mitochondrial DNA* 26: 613–618.
- Jowers JM, Garcia-Mudarra JL, Charles SP, Murphy JC. 2019. Phylogeography of West Indies coral snakes (*Micrurus*): island colonization and banding patterns. *Zoologica Scripta* 48(3): 263–276.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3,059–3,066.
- Koch C, Venegas PJ. 2016. A large and unusually colored new snake species of the genus *Tantilla* (Squamata: Colubridae) from the Peruvian Andes. *PeerJ* 4: e2767.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86(16): 6,196–6,200.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.
- Larkin MA, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21): 2,947–2,948.
- Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 37(2): 581–601.
- Liddle RA. 1946. *The Geology of Venezuela and Trinidad*. Paleontological Research Institution, Ithaca, New York, USA. 890 p.
- Lynch JD. 2015. The role of plantations of the African palm (*Elaeis guineensis* JacQ.) in the conservation of snakes in Colombia. *Caldasia* 37: 169–182.
- Marques OV, Puerto G. 1998. Feeding, reproduction, and growth in the Crowned Snake, *Tantilla melanocephala* (Colubridae), from southeastern Brazil. *Amphibia-Reptilia* 19: 311–318.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *2010 Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, USA. 14 November 2010. IEEE: Piscataway, New Jersey, USA. 115 p.
- Murphy JC. 1997. *Amphibians and Reptiles of Trinidad and Tobago*. Krieger, Malabar, Florida, USA. 245 p.
- Murphy JC, Rutherford MG, Jowers MJ. 2016. The threadsnake tangle: lack of genetic divergence in *Epictia tenella* (Squamata, Leptotyphlopidae): evidence for introductions or recent rafting to the West Indies. *Studies on Neotropical Fauna and Environment* 51: 197–205.

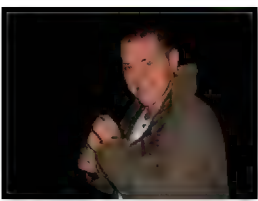
- Murphy JC, Downie JR, Smith JM, Livingstone SM, Mohammed RS, Lehtinen RM, Eyre M, Sewlal JAN, Noriega N, Casper GS, et al. 2018. *A Field Guide to the Amphibians and Reptiles of Trinidad & Tobago*. Trinidad and Tobago Field Naturalists' Club, Port of Spain, Trinidad and Tobago. 336 p.
- Murphy JC, Braswell AL, Charles SP, Auguste RJ, Rivas GA, Borzée A, Lehtinen RM, Jowers MJ. 2019a. A new species of *Erythrolamprus* from the oceanic island of Tobago (Squamata, Dipsadidae). *ZooKeys* 817: 131–157.
- Murphy JC, Salvi D, Santos JL, Braswell AL, Charles SP, Borzée A, Jowers MJ. 2019b. The reduced limbed lizards of the genus *Bachia* (Reptilia, Squamata, Gymnophthalmidae): biogeography, cryptic diversity, and morphological convergence in the eastern Caribbean. *Organisms, Diversity, and Evolution* 19: 321–340.
- Murphy JC, Salvi D, Braswell AL, Jowers MJ. 2019c. Phylogenetic position and biogeography of Three-lined Snakes (*Atractus trilineatus*: Squamata, Dipsadidae) in the eastern Caribbean. *Herpetologica* 75: 247–253.
- Nogueira CC, Argôlo AJ, Arzamendia V, Azevedo JA, Barbo FE, Bérnils RS, Bolochio BE, Borges-Martins M, Brasil-Godinho M, Braz H, et al. 2019. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. *South American Journal of Herpetology* 14(Supplement): 1–274.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice J, Grabowski G. 1991. *The Simple Fool's Guide to PCR*. University of Hawaii Press, Honolulu, Hawaii, USA. 45 p.
- Pindell JL, Kennan L. 2007. Cenozoic kinematics and dynamics of oblique collision between two convergent plate margins: the Caribbean-South America collision in eastern Venezuela, Trinidad, and Barbados. *Transactions of Gulf Coast Section, Society of Economic Paleontologists and Mineralogists (GCSSEPM)* 27: 458–553.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1,253–1,256.
- Ptacek MB, Gerhardt HC, Sage RD. 1994. Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor*. *Evolution* 48: 898–908.
- Pyron RA, Burbrink FT, Wiens JJ. 2013. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. *BMC Evolutionary Biology* 13: 93.
- Rambaut A, Suchard MA, Xie W, Drummond AJ. 2013. Tracer v.1.6.0: MCMC Trace Analysis Tool. BEAST Developers, Edinburgh, Scotland. Available: <http://beast.bio.ed.ac.uk>.
- Ray JM. 2017. *Snakes of Panama: a Field Guide to All Species*. Team Snake Panama, Lexington, Kentucky, USA. 213 p.
- Reynolds RG, Kolbe JJ, Glor RE, López-Darias M, Gómez Pourroy CV, Harrison AS, Queiroz K, Revell LJ, Losos JB. 2020. Phylogeographic and phenotypic outcomes of Brown Anole colonization across the Caribbean provide insight into the beginning stages of an adaptive radiation. *Journal of Evolutionary Biology* 33: 468–494.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1,572–1,574.
- Sabaj MH. 2019. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Version 7.1 (21 March 2019). American Society of Ichthyologists and Herpetologists, Washington, DC, USA. Available: <https://asih.org/standard-symbolic-codes> [Accessed: 1 March 2019].
- Silvestro D, Michelack I. 2012. RaxmlGUI: a graphical front-end for RAXML. *Organisms, Diversity, and Evolution* 12: 335–337.
- Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America* 101: 15,261–15,264.
- Uetz P, Freed P, Hošek J. (Editors). 2020. *The Reptile Database*. Available: <http://www.reptile-database.org> [Accessed: 10 February 2020].
- Vuoto JA. 1998. Se confirma la presencia de *Tantilla melanocephala* (Linnaeus, 1758) (Serpentes: Colubridae) en Pcia. de Entre Ríos-Argentina. *Museo de Ciencias Naturales y Antropológicas "Prof. Antonio Serrano."* Serie Vertebrados (Los Reptiles) 6: 1–12.
- Wilson LD, Mena CE. 1980. Systematics of the *melanocephala* group of the colubrid snake genus *Tantilla*. *San Diego Society of Natural History Memoirs* 11: 5–58.
- Wilson LD. 1992. *Tantilla melanocephala*. *Catalogue of American Amphibians and Reptiles* 547: 1–3.
- Young TP. 2000. Restoration ecology and conservation biology. *Biological Conservation* 92: 73–83.
- Zaher H, Murphy RW, Arredondo JC, Graboski R, Machado-Filho PR, Mahlow K, Montingelli GG, Quadros AB, Orlov NL, Wilkinson M, et al. 2019. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). *PLoS ONE* 14: e0216148.



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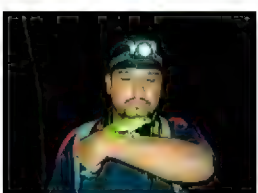
Gilson A. Rivas was born in Caracas, Venezuela. He currently serves as co-editor of the scientific journal *Anartia*, and is a collection manager at the Museo de Biología de la Universidad del Zulia, Maracaibo—a Venezuelan centennial university that began academic activities on 11 September 1891. For over two decades, Gilson has been devoted to the taxonomy and conservation of the neotropical herpetofauna, having authored or co-authored more than 100 academic publications, describing over 30 new species of amphibians and reptiles, and a new genus of dipsadine snake, *Plesiodipsas*. Gilson is the author (with G. Ugueto) of the book *Amphibians and Reptiles of Margarita, Coche, and Cubagua*; and together with M. De Freitas, H. Kaiser, C.L. Barrio-Amorós, and T.R. Barros produced *Amphibians of the Península de Paria: a Pocket Field Guide*. Gilson's research interests are focused on the herpetofauna of the Venezuelan coastal range and insular ecosystems, as well as the influences of invasive species and human development and their impacts on the native fauna.



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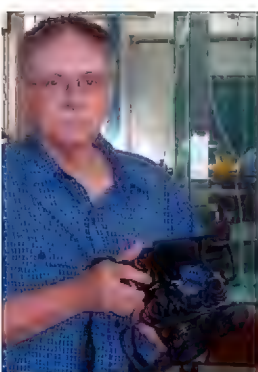
Alvin L. Braswell has a B.S. in Wildlife Biology and an M.S. in Zoology with an emphasis in Herpetology. He retired after 40 years with the Museum of Natural Sciences (MNS, Raleigh, North Carolina, USA) where he transitioned thru the ranks as Collections' Manager for Herpetology and Ichthyology, Curator of Amphibians, Curator for Herpetology, Research Lab Director, and finally Deputy Museum Director for Operations. After 4.5 years of retirement, the joy of being a biologist again, and discovering new and wonderful things, he was called back to serve as Interim Director of the MNS. Now (a year and half later), he is looking forward to re-retirement and having the chance to be a biologist again. Alvin holds an adjunct position at North Carolina State University, where he has co-taught Herpetology from 1996–2013.



Renoir J. Auguste is a Trinidad and Tobago herpetologist. Renoir received his M.Sc. in Biodiversity Conservation from The University of the West Indies, St. Augustine Campus, Trinidad and Tobago, and is interested in the ecology and conservation of amphibians and reptiles. He has conducted herpetological surveys across Trinidad and Tobago for national baseline surveys aimed at improving protected areas, as part of his academic degrees, and also voluntarily with the local environmental NGO *Trinidad and Tobago Field Naturalists' Club*, in which he has held the position of President for three years.



Amaël Borzée is principally interested in behavioral ecology and the conservation of species; and his current research focus is on amphibian breeding behavior and conservation in North East Asia. Amaël has been mostly focusing on treefrogs so far, but is currently expanding his interests to address broader questions for the conservation of multi-species populations over large landscapes, including the use of multiple types of approaches and analytical tools.



John C. Murphy is a naturalist with a focus on snakes. When he is not hiking in the desert or examining specimens in the lab, he is often writing about reptiles. Murphy is a retired science educator who got serious about his lifelong fascination with lizards and snakes in the early 1980s when he and his family made their first trip to Trinidad. The work on Trinidad and Tobago provided valuable lessons that shaped his views of nature and evolution, and today he is still working on the eastern Caribbean herpetofauna. In the 1990s he did some work on homalopsid snakes in Southeast Asia with others from the Field Museum (Chicago, Illinois, USA). He now resides in southeastern Arizona, and is involved in multiple projects on arid habitats and the impacts of climate change on biodiversity. His most recent book is *Giant Snakes, A Natural History* (with co-author Tom Crutchfield). Born and raised in Joliet, Illinois, he first learned about reptiles on his grandfather's farm by watching Eastern Garter Snakes emerge from their winter dens and Snapping Turtles depositing their eggs at the edge of a cattail marsh.

Supplementary Material

Table S1. Primers used in gene fragment amplification, indicating the gene fragments amplified, primers, and references.

Gene	Primer name and sequence	Reference
12S rDNA	12SA 5'-AAACTGGGATTAGATACCCCACTAT -3'	Kocher et al. 1989
12S rDNA	12SB 5'-GAGGGTGACGGGCGGTGTGT -3'	Kocher et al. 1989
16S rDNA	16SL 5'-GCCTGTTTATCAAAAACAT -3'	Palumbi et al. 1991
16S rDNA	16SH 5'-CCGGTCTGAACTCAGATCACGT -3'	Palumbi et al. 1991
cytb	14910 5'-GACCTGTGATMTGAAAAACCAAYCG -3'	Burbrink et al. 2000
cytb	H16064 5'-CTTTGGTTTACAAGAACAATGCTT -3'	Burbrink et al. 2000
cytb	Pacek-L (short) 5'-TGAGGACAAATATCATTCTGAGG -3'	Ptacek et al. 1994
cytb	CB3Xen-H 5'-GGCGAATAGGAARTATCATTC -3'	Goebel et al. 1999
c-mos	S77 5'-CATGGACTGGGATCAGTTATG -3'	Lawson et al. 2005
c-mos	S78 5'-CCTTGGGTGTGATTTTCTCACCT -3'	Lawson et al. 2005

Table S2. Species used in this study, vouchers, and GenBank accession numbers. na: not applicable; asterisks (*) indicate locality identity not confirmed.

Species	Ingroup vouchers	Ingroup country	12S rDNA	16S rDNA	Cytb	C-mos
<i>Chilomeniscus stramineus</i>	na	na	--	--	GQ895856	GQ895800
<i>Chionactis occipitalis</i>	na	na	--	--	GQ895857	GQ895801
<i>Conophis vittatus</i>	na	na	--	--	GQ895862	GQ895806
<i>Conopsis biserialis</i>	na	na	--	--	GQ895860	GQ895804
<i>Pseudoficimia frontalis</i>	na	na	--	--	GQ895886	GQ895827
<i>Sonora aemula</i>	na	na	--	--	JQ265959	JQ265952
<i>Sonora michoacanensis</i>	na	na	--	--	JQ265958	JQ265951
<i>Sonora mutabilis</i>	na	na	--	--	JQ265956	JQ265950
<i>Sonora semiannulata</i>	na	na	--	--	AF471048	AF471164
<i>Stenorrhina freminvillei</i>	na	na	HM565769	--	GQ895889	GQ895830
<i>Drymarchon corais</i>	na	na	HM565758	HM582218	AF471064	AF471137
<i>Drymarchon couperi</i>	na	na	--	--	KP765662	KP765646
<i>Sympholis lippiens</i>	na	na	--	--	GQ895890	GQ895831
<i>Tantilla coronata</i>	LSU H-18896	USA	--	--	KP765669	KP765653
<i>Tantilla gracilis</i>	OMNH41880	USA	--	--	KP765670	KP765654
<i>Tantilla hobartsmithi</i>	MVZ233299	USA	--	--	KP765671	KP765650
<i>Tantilla planiceps</i>	TAPL340	USA	--	--	KP765673	KP765651
<i>Tantilla armillata</i>	FN256487	Guatemala*	KR814613	KR814644	KR814702	KR814681
<i>Tantilla impensa</i>	FN253542	Guatemala*	KR814614	KR814645	KR814688	KR814677
<i>Tantilla vermiformis</i>	FN256027	Guatemala*	KR814615	KR814646	KR814684	KR814665
<i>Tantilla nigriceps</i>	OMNH41890	USA	--	--	KP765672	KP765655
<i>Tantilla relicta1</i>	CAS200845	USA	--	--	AF471045	AF471107
<i>Tantilla relicta2</i>	KW0362	USA	--	--	KP765668	KP765652
<i>Tantilla tjiasmantoi1</i>	CORBIDI:7726	Peru	KY006875	KY006877	--	--
<i>Tantilla tjiasmantoi2</i>	ZFMK:95238	Peru	--	KY006876	--	--
<i>Tantilla melanocephala</i>	MZUSP12976	São Paulo, Brazil	MK209216	MK209331	MK209288	--
<i>Tantilla melanocephala</i>	MNHN 1996.7876	Kourou, French Guiana	AF158424	AF158491	--	--

Table S2 (continued). Species used in this study, vouchers, and GenBank accession numbers. na: not applicable; asterisks (*) indicate locality identity not confirmed.

Species	Ingroup vouchers	Ingroup country	12S rDNA	16S rDNA	Cytb	C-mos
<i>Tantilla melanocephala</i> 1	AMCC101309	Aishalton, Guyana	MT968708	MT968713	MT968722	MT968717
<i>Tantilla melanocephala</i> 2	AMCC101356	Dubulay, Guyana	MT968709	MT968714	MT968723	MT968718
<i>Tantilla melanocephala</i>	UWIZM.2015.18.28	Trinidad	MT968711	MT968716	MT968725	MT968720
<i>Tantilla melanocephala</i>	UWIZM.2016.22.54	Tobago	MT968707	MT968712	MT968721	--
<i>Tantilla melanocephala</i>	MBLUZ 1291	Macuro, Venezuela	MT968710	MT968715	MT968724	MT968719

Table S3. Best partition schemes selected in PartitionFinder for the RaxML and MrBayes analyses, and best models selected in jModeltest for BEAST.

Scheme	Model
PartitionFinder	
12S, 16S rDNA, cytb 1 st codon	GTR+I+G
cytb 2 nd codon	TRN+I+G
cytb 3 rd codon	K81UF+G
c-mos 1 st + 2 nd codon	K80
c-mos 3 rd codon	HKY
jModeltest	
12S rDNA+16S rDNA	TIM2+I+G
cytb	TPM2uf+I+G
c-mos	KHY



The most northeastern record of the Turkish endemic viper, *Pelias barani* (Böhme and Joger, 1984), from northeastern Anatolia: two viper species in a valley

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Abstract.—The Çağlayan Valley is located in Fındıklı district of Rize province, and represents a 34.7-km linear stretch that starts in Fındıklı district and ends in the Yusufeli district borderland of Artvin province in Turkey. Moreover, the valley is the home of two endemic viper species, *Pelias barani* (a Turkish endemic) and *Pelias kaznakovi* (a Caucasus endemic), that are classified by the IUCN as Near Threatened and Endangered, respectively. Here, *Pelias barani* is documented in the Çağlayan Valley for the first time. Due to several threats, most notably a proposed hydroelectric power plant (HPP), these viper species will face increasing challenges such as habitat loss and fragmentation in the near future. Therefore, this study emphasizes that the Çağlayan Valley should be a protected area in terms of these two viper species, and it also shows this area to be the nearest contact zone between *P. barani* and *P. kaznakovi* found thus far.

Keywords. Biodiversity, Çağlayan Valley, contact zone, Reptilia, Rize, Viperidae

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Introduction

Pelias barani, also known as Baran's Viper or Baran's Adder, is a member of family Viperidae and one of several Anatolian vipers. It was first described by Böhme and Joger (1983) based on a female specimen from northwestern Anatolia, with a type locality of 60 km N of Adapazarı, Turkey, at 400 m asl. Many subsequent studies have shown new locality records for *P. barani* from the northwestern and northeastern parts of Anatolia (Baran et al. 1997, 2001, 2005; Franzen and Heckes 2000; Kutrup 2003; Avcı et al. 2004; Kumlutaş et al. 2013; Göçmen et al. 2015; Gül 2015; Mebert et al. 2014, 2015; Gül et al. 2016a,b). In addition, the taxonomic status of *P. barani* has been evaluated in several studies. Joger et al. (1997, 2003) indicated that *P. barani* is a species distinct from *P. berus* in terms of morphological, molecular, and hemipenial data; and this status was also supported by several later studies (Kalyabina-Hauf et al. 2004; Garrigues et al. 2005). As a result, *P. barani* is an endemic species which is only distributed in northwestern and northeastern Anatolia (Göçmen et al. 2015).

Anatolia, which is also known as Asia Minor or the Anatolian peninsula, is home to three of the world's

36 biodiversity hotspots: the Mediterranean basin, Caucasus, and Irano-Anatolian (CEPF 2019; Ergüner et al. 2018), and is the biological crossroads of Asia, Europe, and northern Africa (Ergüner et al. 2018). Northeastern Anatolia in particular is considered as a diversity “hotspot within a hotspot” for the vipers because it includes at least 10 species within a radius of 200 km from Erzurum province (Mebert et al. 2015). Another Caucasus hotspot endemic viper found in the Çağlayan Valley, *Pelias kaznakovi* (Nikolsky, 1909), is classified as Endangered according to the IUCN Red List category and criteria (Gül et al. 2016b). This study reports the most northeastern record of the Turkish endemic *P. barani* (Böhme and Joger 1983), demonstrating that it is another viper species which occupies the Çağlayan Valley.

Materials and Methods

Study area. Fındıklı is a district of Rize Province, Turkey, on the Black Sea coast of northeastern Anatolia, and is also home of two large valleys: the Çağlayan and the Arılı (Selim 2009, 2011). These valleys have particular national and international importance in terms of their unique ecological features (Selim 2011). The

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Fig. 1. Distribution map of *Pelias barani* and *Pelias kaznakovi* (created using ArcGIS 10.4). Red stars: *Pelias barani* from Mebert et al. (2015), blue star: new locality record for *Pelias barani*, yellow stars: known localities of *Pelias kaznakovi*. Inset map indicates previously known localities. Photo by Serkan Gül.

Çağlayan Valley includes areas covering Yusufeli and Arhavi districts of Artvin Province in the southeastern part of the valley, and is 34.7 km in length (Selim 2009). A stream in the valley, known as “Çağlayan Stream,” reaches to the Black Sea and has important influences on various agricultural, settlement, forest, and aquatic ecosystems (Selim 2009). The broader area has a humid subtropical climate with an annual average precipitation of 2,296 mm recorded over the 90-year period of 1928–2018 (TSMS 2019). The study area is largely under the influence of very moist conditions, and summers are usually wetter than winters; annual rainfall is highly variable (TSMS 2019).

Specimen information. One wounded female specimen of *Pelias barani* was found by Tarık Ziya Cengiz in the Çağlayan Valley in Fındıklı, Rize, at 87 m asl on 26 June 2019 (Fig. 1). The specimen was preserved in 96% ethanol and taken to the Zoology Research Laboratory, Recep Tayyip Erdoğan University (Rize, Turkey). Photos of the specimen and its habitat (Fig. 2) were taken by the author, Serkan Gül. The snout-vent length and tail length of the specimen were taken (to the nearest mm) using a ruler and the ventral plates were counted according to Dowling (1951). The terminology used in describing the specimen is in accordance with previous studies (Franzen and Heckes 2000; Avcı et al. 2004; Baran et al. 2005; Kumlutaş et al. 2013; Gül 2015; Gül et al. 2016a,b). All external morphological characters are given in Table 1 along with the data for this species from the relevant literature. Geographic coordinates were collected using the Commander Compass Go 3.9.9 app.

Results and Discussion

Morphological features. The new specimen from a lowland population of the Çağlayan Valley shows little difference in terms of scalation and color pattern from the literature data for this species. The specimen had a total length of 525 mm (head and body length 450 mm;

tail length 75 mm), 144 ventral scales, 32/30 subcaudal scales, and 23 scales on longitudinal rows of the dorsal surface at mid-body. The specimen had two apicals in contact with rostral and two canthals on each side of the head. Loreal scales between the preocular and the post-nasal were 4/4, and there were five scales between the supraoculars. Scale rows between the eyes and upper labials were 1/1 (Table 1).

Color pattern. As described previously by Gül et al. (2016a), the dorsal color pattern of the specimen is almost gray in hue, with a blackish zigzag structure across the dorsal surface (Fig. 2). The head of female specimen is relatively large (Fig. 2A). The ventral color includes many different shades of black, sometimes dark or whitish black, and the ground color of the ventral side is whitish in the anterior part, i.e., the ventral part of the head and neck (Fig. 2B). This whitish color variation continues across both upper labials and lower labials on each side of the head, and to the posterior the ventrals are black with white spots (Fig. 2C–D).

Habitat. *Pelias barani* is usually known to prefer habitats with bush, scrubland, rocky areas, hills, and oak forest (IUCN 2019). The new locality in which the specimen was found has highly transformed anthropogenic post-forest biotopes (Fig. 3A). In fact, *P. barani* actually occupies semi-open landscapes, which fits into the descriptions of the biotopes in other parts of the range, that is, with respect to the openness of the landscape and the combination of light and shade. Additionally, the species richness of trees and shrubs probably play a secondary role. The predominant species at this site include Chestnut (*Castanea sativa*), Oriental Alder (*Alnus orientalis*), European Hornbeam (*Carpinus betulus*), Common Hazel (*Corylus avellana*), various ferns such as *Pteridium tauricum*, and Blackberry (*Rubus fruticosus*) [Fig. 3B]. Other reptile and amphibian species, such as *Bufo bufo* (Pallas, 1814), *Hyla orientalis* (Bedriaga, 1890), *Anguis fragilis* (Linnaeus, 1758), and *Darevskia rudis* (Bedriaga, 1886), probably occupy the same geographic area with *P. barani* (Fig. 3C).

Distribution. *Pelias barani* has a geographic range within the northwestern and northeastern coastal areas of Turkey (Fig. 1). Recently, many new geographic records have appeared in the literature, but a gap remains in terms of its geographic range in the north of Turkey. Gül et al. (2015) showed a geographic record taken from Baran et al. (2001) as the most northeastern point (see Fig. 2 in Gül et al. 2015); however, some authors have highlighted that this geographic record likely represented the “*V. pontica*” (a hybrid of *P. kaznakovi* x *Vipera ammodytes*) collected by Max. Pissié near Chorokhi, Artvin (Zinenko et al. 2013; Göçmen et al. 2015). Therefore, the geographic record presented in this study is very important with respect to establishing a contact

Table 1. Morphometric measurements and scale counts of known specimens based on data from the literature and the new locality. SVL: Snout-vent length, SSO: Scales between supraoculars, EUL: Scale rows between eyes and upper labials, DS: longitudinal rows of dorsal scales. References: 1: Böhme and Joger (1983); 2: Baran et al. (1997); 3: Franzen and Heckes (2000); 4: Baran et al. (2001); 5: Kutrup (2003); 6: Avcı et al. (2004); 7: Baran et al. (2005); 8: Kumlutaş et al. (2012); 9: Gül (2015); 10: Gül et al. (2016a); 11: Göçmen et al. (2015); 12: Current study.

Locality	60 km N of Adapazarı	Camlıhemşin Ardeşen	Dereli Giresun	Dereli Giresun	Fırtına Valley, Ardeşen	Arpagözü Çaykara Trabzon	Arpagözü Çaykara Trabzon	Ballıca, Of, Trabzon	Sugeldi, Of, Trabzon	Çamlık, Vakfikebir, Trabzon	Çamlık, Vakfikebir, Trabzon	Çınarlı, Yomra, Trabzon
Reference	1	2	3	3	3	4	4	5	5	5	5	5
Sex	Female	Female	Female	Male	Female	Female	Female	Male	Male	Female	Male	Female
SVL	472 mm	426 mm	605 mm	545 mm	595 mm	514 mm	395 mm	518 mm	543 mm	186–627 mm	172–197 mm	634 mm
Tail length	68 mm	50 mm	68 mm	73 mm	65 mm	69 mm	51 mm	82 mm	83 mm	32–69 mm	38–46 mm	78 mm
Ventrals	145	145	146	142	145	142	142	140	141	149	140–145	146
Subcaudals	37/37	31/31	31/30	36/36	31/30	28/29	25/28	37	33	30/31	37/38	29
Loreal scales	5/5	5/5	4/4	5/4	11/9	5/5	4/5	5/5	5/no data	4/5	4/5	4/5
Circumoculars	11/12	12/12	9/9	11/10	11/10	9/8	9/9	13/12	10/10	9/10	8/11	11
Apicals	2	no data	no data	no data	no data	1 (2)	2	2	2	2	2	2
Upper labials	10/10	9/9	9/10	9/8	9/9	9/7	9/8	9/9	9/no data	8/9	9/9	9/8
Lower labials	12/12	11/11	11/11	12/12	12/13	9/9	10/11	11/11	11/11	10/11	10/11	10/11
Gulars	no data	4/4	4/4	4/4	4/4	5/4	5/6	no data	no data	no data	no data	no data
Canthals	3/3	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
SSO	5	no data	no data	no data	no data	4	5	no data	no data	no data	no data	no data
EUL	1/1	no data	no data	no data	no data	1/1	1/1	no data	no data	no data	no data	no data
DS	21	21	21	21	23	21	21	21	21	21	21	21

Table 1. continued. Morphometric measurements and scale counts of known specimens based on data from the literature and the new locality. SVL: Snout-vent length, SSO: Scales between supraoculars, EUL: Scale rows between eyes and upper labials, DS: longitudinal rows of dorsal scales. References: 1: Böhme and Joger (1983); 2: Baran et al. (1997); 3: Franzen and Heckes (2000); 4: Baran et al. (2001); 5: Kutrup (2003); 6: Avcı et al. (2004); 7: Baran et al. (2005); 8: Kumlutaş et al. (2012); 9: Gül (2015); 10: Gül et al. (2016a); 11: Göçmen et al. (2015); 12: Current study.

Locality	İkizce Ordu	Geyve Adapazarı	Kozlu Zonguldak	Büyükdere Çayeli Rize	Tektaş village/ Pazar	Tektaş village/ Pazar	Ömerler, Merkez, Bolu	Ömerler, Merkez, Bolu	Çamlıyayla, Bozüyük, Bilecik	Çayyaka, İnegöl, Bursa	Safa, Domanıç, Kütahya	Çağlayan valley/ Fındıklı
Reference	6	7	8	9	10	10	11	11	11	11	11	12
Sex	Male	Female	Male	Female	Female	Female	Male	Male	Female	Male	Female	Female
SVL	415 mm	503 mm	460 mm	600 mm	500 mm	508 mm	434 mm	556 mm	269 mm	455 mm	450 mm	450 mm
Tail length	57 mm	62 mm	60 mm	70 mm	65 mm	62 mm	81 mm	99 mm	42 mm	84 mm	75 mm	75 mm
Ventrals	147	145	145	142	142	143	144	145	147	144	145	144
Subcaudals	34/35	33/33	33/34	29/30	30/28	30/30	43/43+1	43/43+1	33/33+1	42/42+1	30/31	32/30
Loreal scales	4/5	5/5	4/4	5/5	4/4	4/4	4/4	5/5	4/4	4/5	4/5	4/4
Circumoculars	12/13	11/13	11/13	11/12	broken row	broken row	11/11	12/13	10/10	9/10	11/12	10/11
Apicals	2	2	2	2	2	2	2	2	2	2	2	2
Upper labials	9/9	9/9	9/9	9/9	broken row	9/11	9/10	9/9	9/9	9/10	9/8	9/8
Lower labials	12/12	11/11	12/12	11/12	broken row	broken row	10/11	11/11	10/10	10/10	12/11	10/11
Gulars	4/4	no data	4/4	4/4	broken row	4/4	no data	no data	no data	no data	4/4	4/4
Canthals	no	2/2	2/2	2/2	2/2	2/2	no data	no data	no data	no data	2/2	2/2
SSO	4	no data	5	5	5	5	no data	no data	no data	no data	5	5
EUL	1/1	no data	1/1	1/1	broken row	2/2	1/1	1/1	1/1	1/1	1/1	1/1
DS	22	no data	23	21	23	22	21	22	21	21	22	23

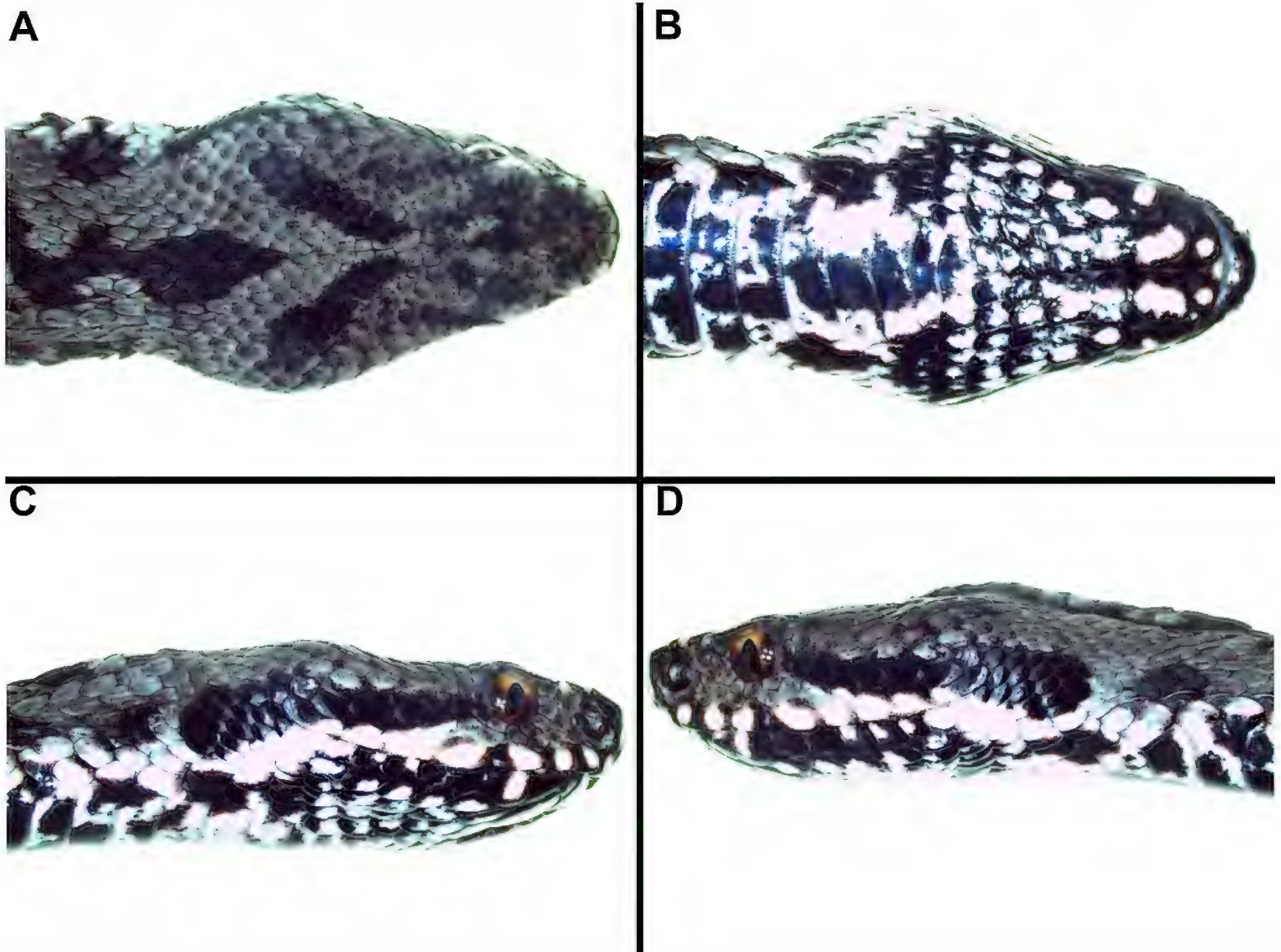


Fig. 2. Dorsal (A) and ventral (B) views of typical head pattern, and each side of the head (C–D) in a female specimen of *Pelias barani* from the Çağlayan Valley, Fındıklı, Turkey. Photos by Serkan Gül.

zone between *P. barani* and *P. kaznakovi*. While Mebert et al. (2015) reported that Işıklı Valley would be the most likely area for a contact zone between them, this study indicates that the Çağlayan Valley is the most likely contact zone between *P. kaznakovi* and *P. barani* (Fig. 4). In addition, based on the finding reported in this study, it appears that the eastern part of the Çağlayan Valley is occupied by *P. kaznakovi* whereas the western part of the Çağlayan Valley is also the habitat of *P. barani* (Fig. 4). Furthermore, with this new finding, the known distance between the *P. barani* and *P. kaznakovi* vipers is reduced from 20.3 km down to only 1.3 km. At the same time, the known distribution of *P. barani* is hereby extended by a distance of up to 19 km northeast from the nearest previously reported site (Mebert et al. 2015; Fig. 4).

An additional reason for these distribution patterns may be related to the Colchic regional characteristics. The Colchis is an ancient region south of the Caucasus Mountains at the eastern end of the Black Sea that is known for many relicts in terms of faunal and floral speciation (Tuniyev 1997). Moreover, the Colchis is a refugial area that explains the presence of relict species in post-glacial dispersal (Tarkhishvili 2014). It seems that the Çağlayan Valley is likely to be the westernmost border of the Colchic refugium in the eastern Black Sea.

Threats and conservation status. In the IUCN Red List, *P. barani* is assigned to the Near Threatened category and criteria. General threats in this area, such as habitat loss due to tourism and recreation areas, hunting and trapping of terrestrial animals for biological resource use, deaths caused by the local people, and road deaths, are potentially threatening for the *P. barani* population (Gül 2015; IUCN 2019). In addition, the increasing human population, and consequent increases of agricultural use, building houses in hitherto unused natural areas, etc., have become additional major threats in the region over the last decade.

However, it seems that the most important threat for both *P. barani* and the overall ecosystem of the valley is hydroelectric power plants (HPP). Although the Çağlayan Valley was declared as a 1st degree priority natural protected area in 2008 by the Trabzon Regional Board for the Protection of Cultural and Natural Heritage, there are still active efforts to build HPP in the valley (Sarıhan 2019). In addition, the Arılı Valley (which is other major valley of Fındıklı) is facing the same problem (DHA 2019). This study indicates that the Çağlayan Valley hosts two endemic viper species, one of which (*P. kaznakovi*) is endemic to the Caucasus hotspot, while the other viper (*P. barani*) is endemic to Turkey. Therefore, the



Fig. 3. Several views of the habitat of *Pelias barani* from the Çağlayan Valley, Fındıklı, Turkey. Photos by Serkan Gül.

construction and operation of the HPP would negatively affect the natural habitats of both species, as well as the other fauna of the river systems and wildlife populations in the valley (Gül et al. 2016a,b). This pursuit of HPP may be a serious problem that leads to decreasing trends of the species populations.

Pelias barani is currently in the IUCN Red List category of Near Threatened (NT), but as stated by Mebert et al. (2015), it will probably qualify for the Vulnerable (VU) category in the near future. In this same valley, *P. kaznakovi* is currently in the Endangered (EN) category, and populations of both species have decreasing trends. Considering all of these factors, clearly the Çağlayan Valley serves as an important habitat for these two viper species of conservation concern. In addition, *Mertensiella caucasica* is an endemic salamander species of the Caucasus hotspot which is also found in this valley and it is listed as Vulnerable by IUCN (Gül et al. 2018). Therefore, the Çağlayan Valley needs to be studied more thoroughly with regard to the diverse herpetofauna and the potential impacts of continuing HPP development.

In conclusion, this study suggests that the Çağlayan Valley should be a protected area, and provides basic information for a conservation action plan for *P. barani* in light of a recent recommendation for the development of a comprehensive, global “Action Plan” for the conservation of vipers (Maritz et al. 2016).

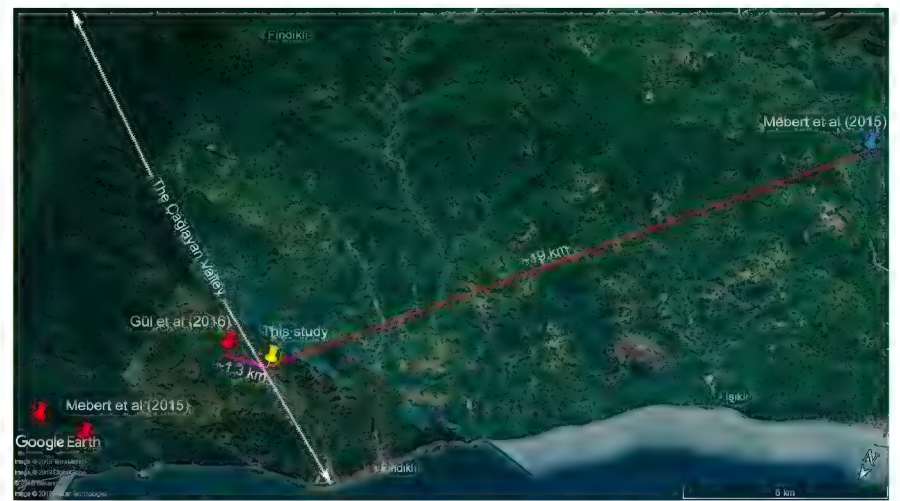


Fig. 4. Proximity of nearest known *Pelias barani* and *Pelias kaznakovi* localities (map generated using Google Earth 7.3.2). Blue pin marker: *Pelias barani* from Mebert et al. (2015), red pin markers: *Pelias kaznakovi* from Mebert et al. (2015) and Gül et al. (2016b), yellow pin marker: new locality record of *Pelias barani* in this study. Distance between blue and yellow pin markers is ~19 km. Note that the short distance between *Pelias barani* and *Pelias kaznakovi* localities in the Çağlayan Valley indicates a potential contact zone.

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Literature Cited

- Avcı A, Üzümlü N, Olgun K. 2004. A new record of *Vipera barani* Böhme and Joger, 1983 (Reptilia, Viperidae) from northeastern Anatolia, Turkey. *Russian Journal of Herpetology* 11: 77–79.
- Baran İ, Tosunoğlu M, Kaya M, Kumlutaş Y. 1997. On the herpetofauna of the vicinity of Çamlıhemşin. *Turkish Journal of Zoology* 21: 409–416.
- Baran İ, Joger U, Kutrup B, Türkozan O. 2001. On new specimens of *Vipera barani* Böhme and Joger, 1983, from northeastern Anatolia, and implications for the validity of *Vipera pontica* Billing, Nilson, and Sattler, 1990 (Reptilia, Viperidae). *Zoology in the Middle East* 23: 47–53.
- Baran İ, Kumlutaş Y, Ilgaz Ç, İret F. 2005. Geographical distributions and taxonomical states of *Telescopus fallax* (Fleischman, 1831) and *Vipera barani* Böhme and Joger, 1983. *Turkish Journal of Zoology* 29: 217–224.
- Böhme W, Joger U. 1983. Eine neue Art des *Vipera berus*-Komplexes aus der Türkei. *Amphibia-Reptilia* 4: 265–271.
- CEPF. 2019. Critical Ecosystem Partnership Fund. Available: <https://www.cepf.net/> [Accessed: 10 July 2019].
- DHA (Demirören Haber Ajansı). 2019. Fındıklı’da HES Protestosu. Available: <https://www.haberturk.com/rize-haberleri/67497211-findiklida-hes-protestosu> [Accessed: 20 July 2019].

- Dowling HG. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Ergüner Y, Kumar J, Hoffman FM, Dalfes HN, Hargrove WW. 2018. Mapping ecoregions under climate change: a case study from the biological ‘crossroads’ of three continents, Turkey. *Landscape Ecology* 34: 35–40.
- Franzen M, Heckes U. 2000. *Vipera barani* Böhme and Joger 1983 aus dem östlichen Pontus-Gebirge, Türkei: Differentialmerkmale, Verbreitung, Habitate. *Spixiana* 23: 61–70.
- Garrigues T, Ferquel CE, Choumet V, Failloux AB. 2005. Molecular phylogeny of *Vipera* Laurenti, 1768 and the related genera *Macrovipera* (Reuss, 1927) and *Daboia* (Gray, 1842), with comments about neurotoxic *Vipera aspis aspis* populations. *Molecular Phylogenetics and Evolution* 35: 35–47.
- Göçmen B, Mebert K, Karış M. 2015. New distributional data on *Vipera (berus) barani* from western and northeastern Anatolia. *Herpetology Notes* 8: 609–615.
- Gül S. 2015. Potential distribution modeling and morphology of *Pelias barani* (Böhme and Joger, 1983) in Turkey. *Asian Herpetological Research* 6: 206–212.
- Gül S, Kumlutaş Y, Ilgaz Ç. 2016a. A new locality record of *Pelias barani* (Böhme and Joger, 1983) from the northeastern Anatolia. *Russian Journal of Herpetology* 23: 319–322.
- Gül S, Kumlutaş Y, Ilgaz Ç. 2016b. Predicted distribution patterns of *Pelias kaznakovi* (Nikolsky, 1909) in the Caucasus Hotspot with a new locality record in Turkey. *Russian Journal of Herpetology* 23: 224–230.
- Gül S, Kumlutaş Y, Ilgaz Ç. 2018. Potential distribution under different climatic scenarios of climate change of the Vulnerable Caucasian Salamander (*Mertensiella caucasica*): a case study of the Caucasus Hotspot. *Biologia* 73: 175–184.
- IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-2. Available: <https://www.iucnredlist.org> [Accessed: 25 July 2019].
- Joger U, Lenk P, Baran İ, Böhme W, Ziegler T, Heidrich P, Wink M. 1997. The phylogenetic position of *Vipera barani* and of *V. nikolskii* within the *Vipera berus* complex. Pp. 185–194 In: *Herpetologia Bonnensis*. Editors, Böhme W, Bischoff W, Ziegler T. Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, Germany. 414 p.
- Joger U, Kalyabina-Hauf SA, Schweiger S, Mayer W, Orlov NL, Wink M. 2003. Phylogeny of Eurasian *Vipera* (subgenus *Pelias*). Abstract 77 In: *Programme and Abstracts. 12th Ordinary General Meeting of the S.E.H., St. Petersburg, Russia, 12-16 August 2003*. Societas Europaea Herpetologica, Bonn, Germany. 200 p.
- Kalyabina-Hauf S, Schweiger S, Joger U, Mayer W, Orlov N, Wink M. 2004. Phylogeny and systematics of adders (*Vipera berus* complex). *Mertensiella* 15: 7–15.
- Kumlutaş Y, Sözen M, Ilgaz Ç. 2013. New record of the rare *Vipera barani* Böhme and Joger, 1983. *Herpetozoa* 25: 183–188.
- Kutrup B. 2003. The identification of new specimens of *Vipera* from Trabzon, Turkey, with affinities to *Vipera barani* and *V. pontica*. *Herpetological Review* 43: 28–31.
- Maritz B, Penner J, Martins M, Crnobrnja-Isailović J, Spear S, Alencar LR, Sigala Rodriguez J, Messenger K, Clark RW, Soorae P, et al. 2016. Identifying global priorities for the conservation of vipers. *Biological Conservation* 204: 94–102.
- Mebert K, İğci N, Göçmen B, Ursenbacher S. 2014. Vipern der Nordost-Türkei: Genfluss und Umweltfaktoren zwischen den Taxa des *Vipera barani-kaznakovi-darevskii*-Komplexes. *Elaphe* 49: 58–67.
- Mebert K, Göçmen B, İğci N, Oğuz MA, Karış M, Ursenbacher S. 2015. New records and search for contact zones among parapatric vipers in the genus *Vipera* (*barani*, *kaznakovi*, *darevskii*, *eriwanensis*), *Montivipera* (*wagneri*, *raddei*), and *Macrovipera* (*lebetina*) in northeastern Anatolia. *The Herpetological Bulletin* 133: 13–22.
- Sarıhan Z. 2019. Fındıklı’da doğal sit alanına hes planlıyorlar. Available: <http://www.findiklihaber.com/findikli/findikli-da-dogal-sit-alanina-hes-planliyorlar-h1999.html> [Accessed: 20 July 2019].
- Selim S. 2009. Doğu Karadeniz yöresi, Fındıklı ilçesi, Çağlayan ve Arılı vadi ekosistemleri ile bu ekosistemlerin etkileşimleri üzerine araştırmalar. Masters Thesis, Institute of Science, Ege University, İzmir, Turkey.
- Selim S. 2011. Akarsu Vadisindeki İnsan Kaynaklı Faaliyetlerin Ekosistem Bütünlüğüne Olası Etkileri: Çağlayan Vadisi Örneği. *Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü Dergisi* 15: 94–101.
- Tarkhnishvili D. 2014. *Historical Biogeography of the Caucasus*. Nova Science Publishers, New York, New York, USA. 234 p.
- TSMS. 2019. Turkish State Meteorological Service, Ankara, Turkey. Available: <https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=RIZE> [Accessed: 28 July 2019].
- Tuniyev BS. 1997. About exact borders of the Colchis biogeographical province. *Russian Journal of Herpetology* 4: 182–185.
- Zinenko O, Stümpel N, Mazanaeva LF, Shiryaev K, Nilson G, Orlov NL, Tuniyev BS, Ananjeva NB, Murphy R, Joger U. 2013. The puzzling phylogeny of the *Vipera kaznakovi*-complex. Pp. 197 In: *Programme and Abstracts, 17th European Congress of Herpetology (SEH), 20–27 August 2013, Veszprém, Hungary*. Societas Europaea Herpetologica, Bonn, Germany. 332 p.



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Rediscovery of *Oligodon catenatus* (Blyth, 1854) (Squamata: Colubridae) from India

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Abstract.—The poorly known Assam Kukri Snake, *Oligodon catenatus* (Blyth, 1854), in the *Oligodon dorsalis* group, is here reported from Mizoram State, northeastern India, based on a single male specimen. This report extends the distributional range of the species. This specimen is only the second one collected from India, and it is very important as the holotype of the species has been lost. A brief description of the new specimen is presented.

Keywords. Assam Kukri Snake, distribution, first record, Mizoram, moist deciduous forest, Tam Dil, wetland

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Introduction

The Assam Kukri Snake, *Oligodon catenatus* (Blyth, 1854), is a poorly known species in India. The type specimen was collected from “Asám” (which currently spans seven states) in northeastern India by Blyth (Smith 1943), but has been lost from the Asiatic Society Museum, Kolkata (Sclater 1891). The distribution of this species includes Myanmar, Laos, Vietnam, China, and possibly Cambodia (Green 2010), and the species has been found at elevations of around 700–1,000 m asl (Zhao et al. 1998). While it has been considered rare since the day of its description (Sharma 2019), the species has not been reported from India in recent years. In Vietnam, it has been collected from evergreen secondary forest (Pham et al. 2014), but very little is known about its ecology (Gong et al. 2002). The nearest known distribution record outside of India is in Nam Tamai Valley near the Tibetan border, Kachin Hills, Myanmar (Smith 1943).

Oligodon catenatus had been a synonym of *O. eberhardti* (e.g., Zhao 2006a,b), but was recently removed from synonymy (see Ziegler et al. 2007; Thy and Nguyen 2012). *Oligodon catenatus* is very similar to, has been confused with, *O. eberhardti* (Thy and Nguyen 2012), but they differ by the absence of the loreal in *O. catenatus* (Pham et al. 2014). In fact, Vassilieva (2015) noted that the absence of a loreal

scale is rare among Kukri snakes. Among the 22 species of *Oligodon* known from Indo-China, this character is found only in three species: *O. catenatus* (Blyth 1854), *O. annamensis* (Leviton 1953), and *O. lacroixi* (Angel and Bourret 1933); and it is facultatively absent in both *O. mouhoti* (Boulenger 1914) and *O. macrurus* (Angel 1927). Before this current report, there had been no previous records of the species from Mizoram (Mathew 2007; Lalremsanga et al. 2011).

Methods

A single male specimen was found dead in a field, and collected on 4 June 2019 from a forest pathway near Tamdil National Wetland (23°44'20"N, 92°57'06"W; elevation 760 m asl), Aizawl District, Mizoram, 64 km E from Aizawl, the capital of Mizoram State, northeastern India. The specimen was fixed in 4% formalin, transferred to 70% ethanol, and deposited in the Departmental Museum of Zoology, Mizoram University, India (as specimen MZMU 1446). Measurements were taken with a slide caliper to the nearest 0.1 mm, except for snout-vent length (SVL) and tail length (TaL), which were measured with a ruler to the nearest 1 mm. The ventral scales were counted according to Dowling (1951), and dorsal scale rows are given at one head length behind the head, at mid-body, and at one head length before the vent. The terminal

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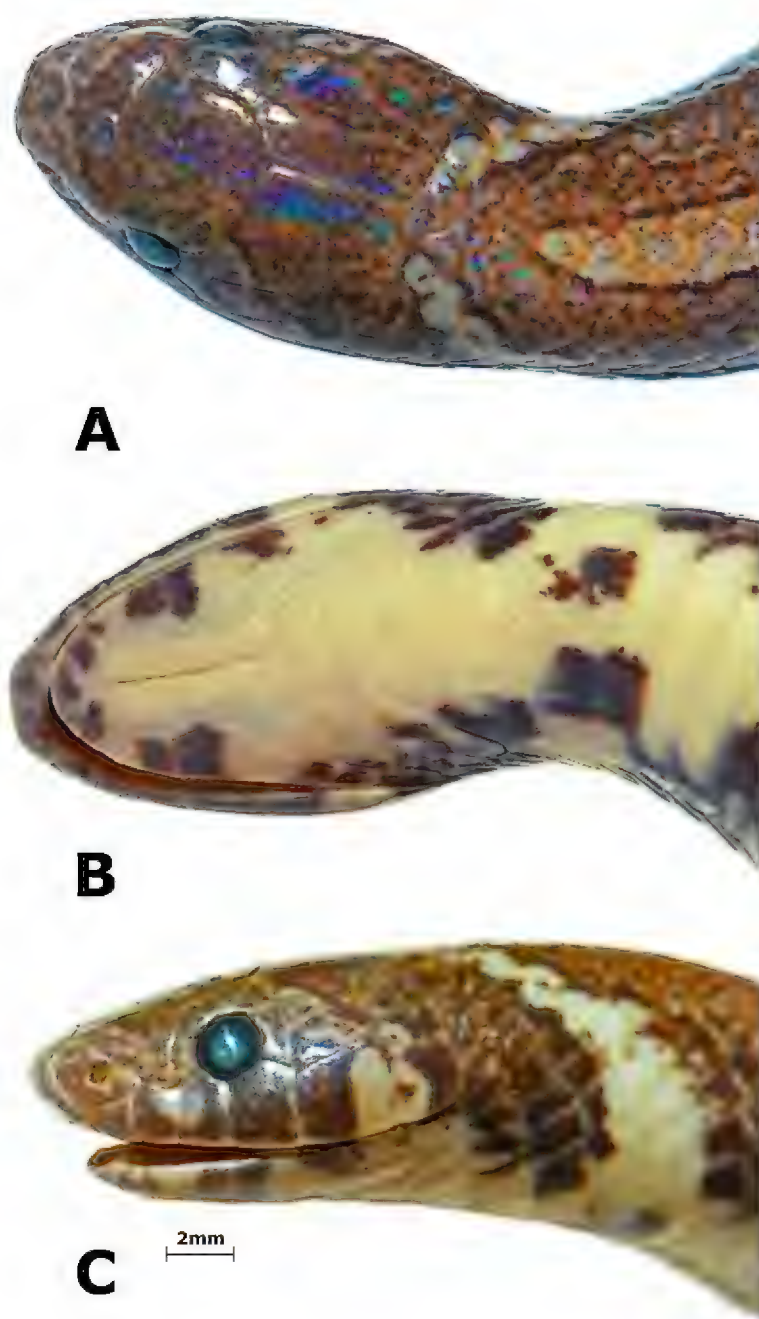


Fig. 1. Head of *Oligodon catenatus* in dorsal (A), ventral (B), and lateral (C) views. Photos by Lalbiakzuala, Melvin Selvan, and Nilanjan Mukherjee.

scute is excluded from the number of subcaudals. Morphometric characters and pholidosis measurements are given in Table 1.

Abbreviations. MZMU: Museum of Zoology Department, Mizoram University, Aizawl, India; DST-SERB: Department of Science and Technology, Science and Engineering Research Board, Government of India; SVL: snout-vent length; TaL: tail length; TL: total length; TaL/SVL: ratio tail length/snout-vent length; HL: head length; HW: head width; DSR: number of dorsal scale rows (at the three positions as described above); VEN: ventral scales; IF: infralabials; SL: supralabials; PosOc: postocular scale; PreOc: preocular scales; SC: subcaudal scales; ATem: anterior temporal scale; PTem: posterior temporal; LOR: loreal scale; SL2: number of supralabials touching eye; EYD: eye diameter; END: eye-nostril distance; WSN: width of snout; LSN: length of snout.

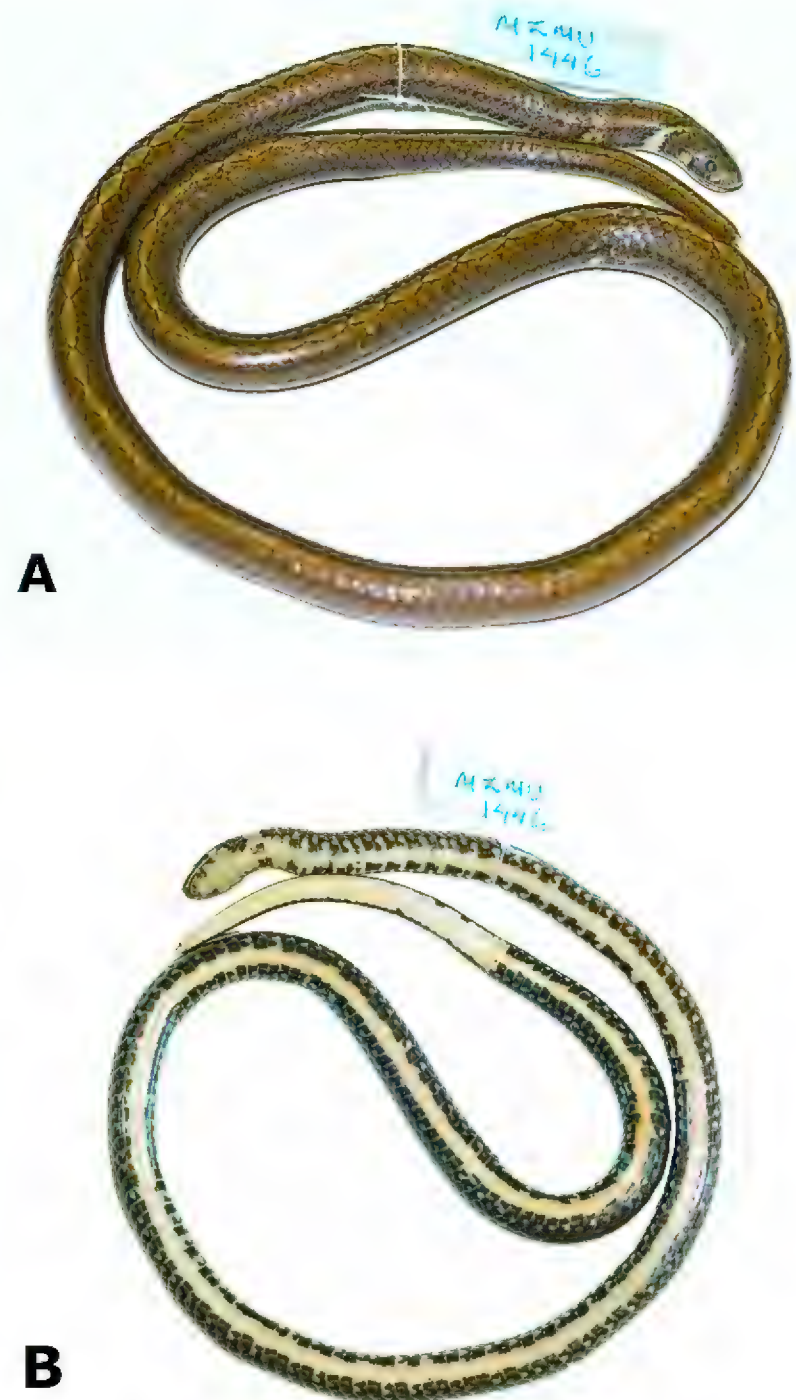


Fig. 2. The dorsal (A) and ventral (B) views of *Oligodon catenatus* in preservation. Photos by Lalbiakzuala, Melvin Selvan, and Nilanjan Mukherjee.

Results and Discussion

The specimen, an adult male, shows an injury ventro-laterally between the 8th and 9th ventral scales, and the opening reaches up to the middle of the ventral region. The body of the snake is cylindrical and it has a small, indistinct head (Fig. 1); small eyes with round pupil; nape with incomplete cross bar; dorsum (Fig. 2) with a chain of 62 continuous diamond-shaped vertebral patterns from the anterior neck region up to the vent, and continuing with a longitudinal stripe to tail-tip; in preservation, outer end of ventral shields with dark squarish spots; mid-ventral with plain pale lemon color from chin to tail tips; ventral tail roughly immaculate, and ends with pointed tip. The loreal scale is absent on both sides; 13 rows of dorsal scales at mid-body; anal shield divided; six supralabials with 3rd and 4th entering orbit on both sides, half of the posterior of 3rd supralabial to half of the anterior of 5th supralabial with marbled

Table 1. Morphometric and pholidosis data of *Oligodon catenatus* specimen found in India in 2019 (voucher number MZMU 1446). See text for abbreviations.

Attribute	State or value
Sex	Male
Collection locality	Tamdil National Wetland, Mizoram, India
Collectors	Lalremsanga and Lalbiakzuala
Date of collection	4 June 2019
EYD	1.96 mm
END	2.78 mm
WSN	3.53 mm
LSN	0.93 mm
HW	8.16 mm
HL	10.82 mm
TaL	64 mm
SVL	480 mm
TaL/SVL	0.13
VEN	203
DSR	13-13-13
IF	6
SL	6
SL2	3 rd and 4 th
ATem	1
PTem	2
PosOc	1
PreOc	1
LOR	Absent
SC	37

dark color, anterior part of the snout is mottled with dark patches; six infralabials; one anterior temporal; 37 paired subcaudals. This description agrees with those given by other workers, except for the ventrals here being 203 vs. 186–196 (Smith 1943) and 179–184 (Pham et al. 2014), but in agreement with the pooled sex ventral range 179–212 (Pauwels et al. 2002; Das 2010).

This collection of the Assam Kukri Snake, *Oligodon catenatus*, from Tam Dil National Wetland is the first record for Mizoram State, and constitutes only the second specimen from India (Fig. 3), since no other specimen has been reported from India after the description of this species in 1854. The holotype had apparently disappeared before the collections of the Asiatic Society were transferred to the Indian Museum (Sclater 1891). The present specimen was collected from an altitude of 760 m asl, which is in the ranges reported by Zhao et al. (1998) and Gong et al. (2007). The present specimen, MZMU 1446, represents the only known specimen for India. The fact that no other specimen has been found in India in over 165 years may be due to the scarcity of surveys and/or population declines due to habitat defragmentation.

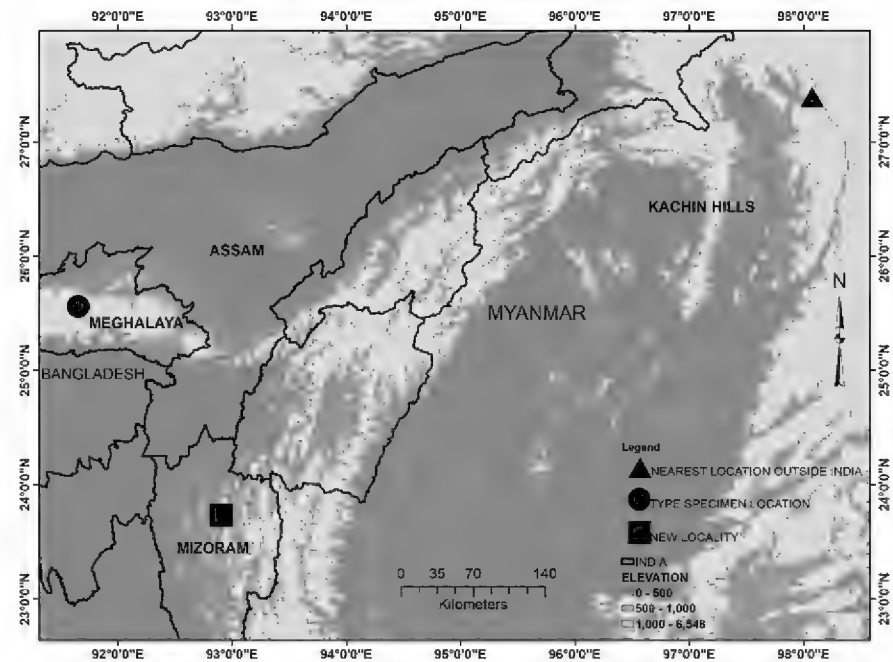


Fig. 3. Map showing the type locality of *Oligodon catenatus* in Khasi Hills, India (solid dark circle); the nearest locality outside India in Kachin Hills, Myanmar (solid dark triangle); and the new locality in Mizoram, India (solid dark square).

The collection site (Fig. 4) is a wetland that is covered by the Natural Wetland Conservation Programme 2006–2007 of the Government of India, in which an area of 285 ha is protected for wetland functions (Anon 2007). It is surrounded by tropical evergreen and moist deciduous forest dominated by *Schima wallichii*, *Chukrasia tabularis*, *Gmelina arborea*, *Artocarpus* sp., *Dendrocalamus* sp., *Albizia* sp., *Morus* sp., and others. From this wetland, new state reports of *Protobothrops mucrosquamatus* (Lalremsanga et al. 2017) and *Leptolalax tamdil* (Sengupta et al. 2010) were recently described. This location is an important tourist attraction and a holiday resort.

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Literature Cited

Angel F. 1927. Liste des reptiles et des batracien rapportés d’Indo-Chine par M.P. Chevey. Description d’une variété nouvelle de *Simotes violaceus* Cantor.

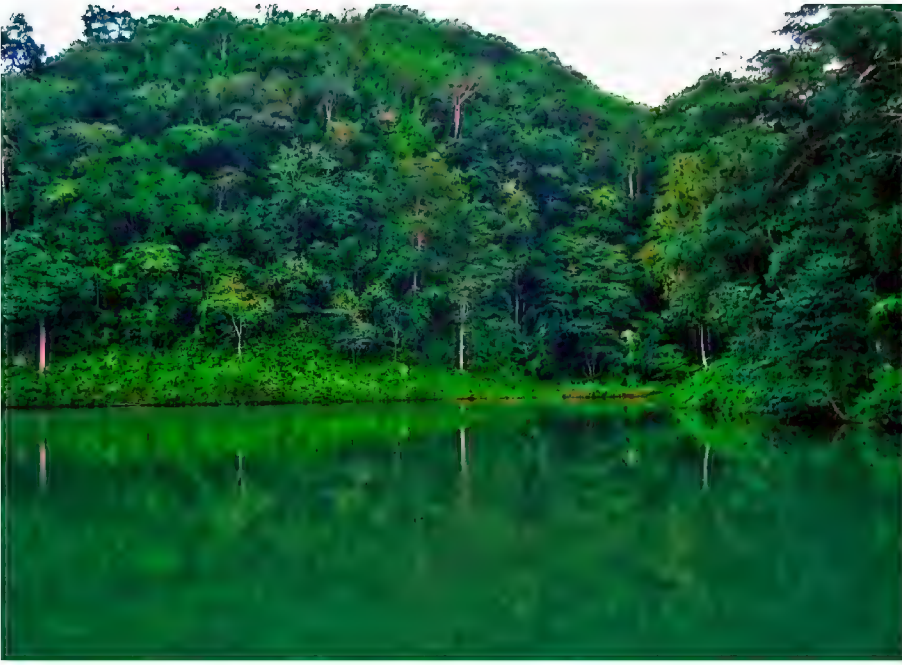
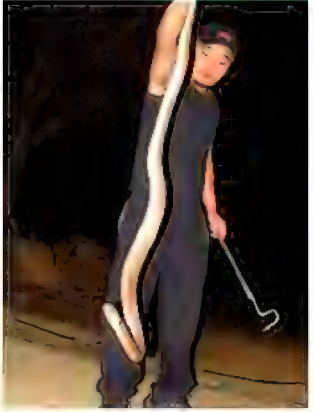


Fig. 4. View of the natural habitats of *Oligodon catenatus* in Tam Dil National Wetland. Photo by H.T. Lalremsanga.

- Bulletin du Muséum national d'histoire naturelle* 33: 496–498.
- Angel F, Bourret R. 1933. Sur une petite collection de serpents du Tonkin. Descriptions d'espèce nouvelles. *Bulletin de la Société zoologique de France* 58: 129–140.
- Anon. 2007. *Conservation of Wetlands in India: a Profile (Approach and Guidelines)*. Conservation Division-1, Ministry of Environment and Forests, Government of India, New Delhi, India. 56 p.
- Blyth E. 1854. Notices and descriptions of various reptiles, new or little known. *The Journal of the Asiatic Society of Bengal* 23(3): 287–302.
- Boulenger GA. 1914. Descriptions of new reptiles from Siam. *The Journal of the Natural History Society of Siam* 1: 67–76.
- Das I. 2010. *A Field Guide to the Reptiles of South-East Asia*. New Holland Publishers (UK) Ltd., London, United Kingdom. 377 p.
- Dowling HG. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Gong SP, Markus A, Zhang YY, Zhong GF, Zeng JD. 2007. A new record of *Oligodon catenatus* in Guangdong Province, China. *Chinese Journal of Zoology* 42(6): 149–150.
- Green MD. 2010. Molecular phylogeny of the snake genus *Oligodon* (Serpentes: Colubridae), with an annotated checklist and key. M.S. Thesis, Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada. 169 p.
- Lalremsanga HT, Sailo S, Chinliansiam. 2011. Diversity of snakes (Reptilia: Squamata) and role of environmental factors in their distribution in Mizoram, Northeast India. Pp. 265–268 In: *Advances in Environmental Chemistry, September 2018*.
- Lalremsanga HT, Lalbiakzuala, Lalrinsanga. 2017. Geographic distribution: *Protobothrops mucrosquamatus* (Brown Spotted Pitviper). *Herpetological Review* 48(1): 131.
- Leviton AE. 1953. A new snake of the genus *Oligodon* from Annam. *Journal of the Washington Academy of Sciences* 43(12): 422–424.
- Mathew R. 2007. Reptilia. Pp. 545–577 In: *Fauna of Mizoram*. Records of the Zoological Survey of India, State Fauna Series, 14. Zoological Survey of India, Kolkata, India. 691 p.
- Pauwels OSG, Wallach V, David P, Chanhom L. 2002. A new species of *Oligodon* (Serpentes, Colubridae) from southern peninsular Thailand. *Natural History Journal of Chulalongkorn University* 2(2): 7–18.
- Pham AV, Nguyen SLH, Nguyen TQ. 2014. New records of snakes (Squamata: Serpentes) from Son La Province, Vietnam. *Herpetology Notes* 7: 771–777.
- Sclater WL. 1891. Notes on a collection of snakes in the Indian Museum, with descriptions of several new species. *The Journal of the Asiatic Society of Bengal* LX: 230–250.
- Sengupta S, Sailo S, Lalremsanga HT, Das A, Das I. 2010. A new species of *Leptolalax* (Anura: Megophryidae) from Mizoram, North-eastern India. *Zootaxa* 2406: 57–68.
- Sharma V. 2019. Assam Kukri Snake (*Oligodon catenatus* Blyth 1854). Available: <http://indiansnakes.org/content/assam-kukri-snake> [Accessed: 24 July 2019].
- Smith MA. 1943. *The Fauna of British India, Ceylon and Burma including the Whole of the Indo-Chinese Sub-region, Reptilia and Amphibia. Volume 3 Serpentes*. Taylor and Francis, London, United Kingdom. 583 p.
- Thy N, Nguyen TQ. 2012. *Oligodon eberhardti*. The IUCN Red List of Threatened Species 2012: e.T191932A2017150.
- Uetz P, Freed P, Hosek J. (Editors). 2018. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 23 July 2019].
- Vassilieva AB. 2015. A new species of the genus *Oligodon* Fitzinger, 1826 (Squamata: Colubridae) from coastal southern Vietnam. *Zootaxa* 4058(2): 211–226.
- Zhao EM. 2006a. *The Snakes of China. Volume I*. Anhui Science and Technology Publishing House, Hefei, China. 372 p. [in Chinese].
- Zhao EM. 2006b. *The Snakes of China. Volume II*. Anhui Science and Technology Publishing House, Hefei, China. 280 p. [in Chinese].
- Zhao EM, Huang MH, Zong Y. 1998. *Fauna Sinica: Reptilia, Volume 3. Squamata, Serpentes*. Science Press, Beijing, China. 570 p.
- Ziegler T, Hendrix R, Thanh VN, Vogt M, Forster B, Kien DN. 2007. The diversity of a snake community in a karst forest ecosystem in the central Truong Son, Vietnam, with an identification key. *Zootaxa* 1493: 1–40.



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A reevaluation of records of Sandveld lizards, *Nucras* Gray, 1838 (Squamata: Lacertidae), from northern Namibia

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Abstract.—Data relating to the Sandveld lizards (*Nucras*) occurring in Namibia, southwest Africa are reviewed. In particular, we investigated records of *N. holubi*, a chiefly southeastern African species, and attempted to identify recently collected material that could not be assigned to any species currently recognized in Namibia. A phylogenetic analysis of *Nucras* based on three mitochondrial markers revealed a deep divergence between Namibian *Nucras holubi* and two presumably conspecific clades from Limpopo Province, South Africa. In addition, the coloration pattern and scalation of the Namibian material differ from those of the eastern forms, supporting its recognition as a separate species. The name *Nucras damarana* Parker, 1936, long relegated to the synonymy of *N. holubi*, is here resurrected for this apparently endemic northern Namibian species. *Nucras damarana* is restricted to the Kunene, Omusati, Oshikati, Kavango, and Otjozondjupa regions of north-central Namibia. A distinctive specimen of *Nucras* from near Ruacana in the Kunene Region was identified as allied to *Nucras broadleyi*, a species recently described from southwestern Angola, on the basis of genetic data, although it differs substantially in color pattern. With the addition of *N. aff. broadleyi* and the resurrected *N. damarana* to its fauna, as well as the removal of *N. holubi* from the nation's species list, four species of *Nucras* are confirmed to be present in Namibia. Although the conservation status of *N. damarana*, *N. tessellata*, and *N. intertexta* is Least Concern, the uncertain taxonomic status of *N. aff. broadleyi* precludes a meaningful threat assessment.

Keywords. Distribution, endemism, *Nucras damarana*, *Nucras broadleyi*, *Nucras holubi*, phylogeny, taxonomy

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Introduction

Three species of Sandveld lizards, *Nucras* (Lacertidae), are currently regarded as occurring in Namibia: *N. intertexta* (Smith, 1838), *N. tessellata* (Smith, 1838), and *N. holubi* (Steindachner, 1882) (Branch 1998; Griffin 2003; Herrmann and Branch 2013). Paradoxically, the first species recorded from what is now Namibia was a species that does not occur in the country. Boulenger (1887) reported *N. delalandii* (= *N. lalandii*), from “Damaraland,” an area corresponding to most of interior central and northern Namibia. This record was repeated by Werner (1910), Boulenger (1910), and Sternfeld (1911a), with the last author adding a second record from Gobabis. However, both Hewitt (1910) and Boulenger (1917) questioned the locality, the latter noting that the

specimen had been obtained by purchase in 1865 without an indication of the collector. Indeed, this specimen is part of a collection of specimens obtained from W. Stevens, all from Damaraland (BMNH 65.6.18.1–13, 65.8.28.1–31). In fact, it is likely that none of this material originated in the area then referred to as Damaraland. In addition to *N. lalandii*, the collection included specimens of *Afrogecko porphyreus* (BMNH 65.6.18.11), which is endemic to the southern and southwestern portions of the Cape provinces; *Philothamnus natalensis* (BMNH 65.6.18.1) and *P. hoplogaster* (BMNH 65.6.18.2), which are both limited to eastern southern Africa; and the types of *Chamaeleon damaranum* = *Bradypodion damaranum* (BMNH 65.6.18.4–5), which is endemic to the south coast of the Western and Eastern Cape provinces. Boulenger (1920) presented data for a specimen of *N. delalandii* from “Great Namaqualand,” but by the

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time of the subsequent genus revision, this species was recognized as being restricted to the eastern portions of the subcontinent (FitzSimons 1943).

Fischer (1888) first recorded *N. tessellata* from between Aus and Bethanie, with further records given by several authors: Boettger (1893, 1894: Bethanien, Rehoboth, and environs), Werner (1910: Okahandja and Kubub, 1915: Usakos), Sternfeld (1911a,b: Deutsch Südwest-Afrika), and Methuen and Hewitt (1914: Kraikluft and between Nakeis and Groendoorn). However, subsequent authors (e.g., Broadley 1972; FitzSimons 1943; Mertens 1955) interpreted some of these (Okahandja and Usakos) records as representing other species. FitzSimons (1943) considered *N. tessellata* to be widespread, although his knowledge of it was based on the published German colonial records noted above. Mertens (1955) likewise reported older localities but noted that he knew the species with certainty only from Rehoboth. Broadley (1972) accepted the same southern Namibian localities as Mertens (1955), i.e., as far north as the area around Rehoboth, but added a record 20 km N of Rosh Pinah. The current concept of the species limits its Namibian distribution to the far south and southeast of the country, thereby excluding most of the historical records (Branch 1998), although Rehoboth area records were accepted by Visser (1984) and Griffin (2003).

Hoesch and Niethammer (1940) first reported *Nucras intertexta* from Namibia from the area of the Waterberg. FitzSimons (1943) further recorded it from Sandfontein near Gobabis, whereas Mertens (1955) added Okahandja and Boettger's (1893) record of *N. tessellata* from Bethanie to the *N. intertexta* records. Broadley (1972) added many records from northwestern Namibia and considered *N. intertexta* to be widespread across the northern half of Namibia (plus Bethanie), most of Botswana, northern South Africa, southern Zimbabwe, and southern Mozambique. The Bethanie record has since been considered to be an error by Branch (1998), but the northern Namibian records form a diagonal from the Omaheke Region northwest to the Angolan border in the Kunene Region (Branch 1998; Visser 1984).

Nucras intertexta holubi was first recorded from Namibia by FitzSimons (1943) based on Werner's (1910, 1915) earlier records of *N. tessellata* from Okahandja and Usakos, respectively, and new records from Outjo, Kaoko Otavi, and Otjitondua. Mertens (1955) listed the same records but called into question both the identity of the specimens and the validity of the taxon. Broadley (1972) included this form in his concept of *N. taeniolata ornata*, with records from Kombat, Opuwo, Ombombo, Oshakati, Kaoko Otavi, Otjitondua, Otjivakandu, Outjo, and Sissekab, all in northern Namibia in the current Kunene, Oshana, and Otjozondjupa regions, in addition to a single southern record from Stamprietfontein (2418AD, Hardap Region). Jacobsen

(1989) subsequently elevated *N. ornata* to full species and treated *N. t. holubi* as subspecifically distinct within *taeniolata*, and Bates (1996) later demonstrated the specific validity of *N. holubi*, although neither of them explicitly reevaluated the Namibian "*holubi*." Branch (1998) mapped the distribution in Namibia and apparently treated the northern and western records as referable to *N. intertexta*, but without comment. Visser (1984) and Griffin (2003), however, recognized as valid records from as far northwest as Opuwo.

A fourth taxon, *N. intertexta damarana*, was described by Parker (1936) from Sissekab in north central Namibia. It was considered a valid subspecific form with a small area of endemism by both FitzSimons (1943) and Mertens (1955, 1971). However, Broadley (1972) included it, along with *N. i. holubi*, in the synonymy of *N. taeniolata ornata*. This nominal taxon has not been accepted as valid since, nor has its status been reevaluated, although Branch et al. (2019a) suggested that the name *damarana* was applicable to northern Namibian *N. holubi*, without commenting on its validity.

As currently construed, both *Nucras tessellata* and *N. intertexta* have broad distributions, the Namibian portions of which are contiguous with the rest of their respective ranges (Branch 1998; Visser 1984). However, *Nucras holubi* is currently recognized as having a disjunct distribution in southern Africa. The main area of occurrence extends from about 31°S in the northern Eastern Cape Province of South Africa, to the north and east to include the central and northeastern provinces of South Africa, Eswatini (formerly Swaziland), eastern Botswana, Zimbabwe, southern Malawi, and almost certainly parts of Mozambique; while the second area is in north central Namibia (Bourquin 2004; Branch 1998; Burger 2014; De Waal 1978; Griffin 2003; Jacobsen 1989). The large gap between these two areas (> 900 km) has long suggested to herpetologists that the status of the Namibian population required further investigation.

Here morphological and molecular data from northern Namibian specimens of *Nucras* were used to reevaluate the status of *N. holubi* and *N. damarana* in the country. The possibility that a recently described Angolan species might also occur in Namibian territory was also investigated. The *Nucras* in southwestern Angola had variously been referred to either *N. tessellata* (Bocage 1895), *N. t. taeniolata* (Boulenger 1910), or *N. intertexta holubi* (Boulenger 1917, 1920), and was considered to represent a new species by Broadley (1972; see review therein). Branch et al. (2019a) stabilized the situation in Angola by describing the species known from Namibe, Huila, and Cunene provinces in southwestern Angola as *Nucras broadleyi*, the southernmost record of which is from Donguena, Cunene Province (-17.01667, 14.71667), only 42 km north of the Namibian border.

Materials and Methods

Specimens. Standard institutional codes used in this paper are: CAS (California Academy of Sciences, San Francisco, California, USA), MCZ (Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA), NHMUK (The Natural History Museum, London, United Kingdom), NMB (National Museum, Bloemfontein, South Africa), NMNW (National Museum of Namibia, Windhoek, Namibia), NMW (Naturhistorisches Museum Wien, Vienna, Austria), PEM (Port Elizabeth Museum, Port Elizabeth, South Africa), SAM (Iziko, South African Museum, Cape Town, South Africa), DNMNH (Ditsong National Museum of Natural History, Pretoria, South Africa), and ZFMK (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany). Additional tissue samples were derived from the collections of Vincent Egan (LMH), Marius Burger (MB/MBUR), Michael Cunningham (MH), and Raymond B. Huey (RBH).

Morphology. The following mensural features were recorded to the nearest 0.1 mm using digital calipers: SVL (snout-vent length), TrW (trunk width), TailL (tail length), TailW (maximum tail width), AGL (axilla-groin length), HumL (humerus length), ForeL (forearm length), FemL (femur length), CrusL (shank length from knee to heel), PesL (pes length from heel to tip of 4th toe), HeadL (head length), HeadW (head width), HeadD (Head depth), CSn (collar-snout length), OrbD (eye diameter = width of eye), NEye (nostril to eye distance), EyeE (eye to ear distance), EarD (maximum height of ear opening), and EarW (maximum width of ear opening). Details of pholidosis including head scalation, as well as femoral pore disposition, were also recorded. Except in the case of MCZ R-190201 (see below), scalation and pore data were collected unilaterally, for head scalation from the right side of the body and femoral pores from the left side of the specimen only. Morphological data are presented in Table 1. Additional data for *N. holubi* were derived from Broadley (1972) and Jacobsen (1989). Comparisons were made with all other described species of *Nucras* based on material listed in Bauer et al. (2019) and from literature sources (e.g., Branch et al. 2019a; Broadley 1972).

Molecular data. New sequence data were generated for specimens of *Nucras* sp. ($n = 1$) and *N. holubi* from South Africa ($n = 4$) and Namibia ($n = 1$), and were combined with sequence data generated for a previous phylogenetic study on South African *Nucras* (Bauer et al. 2019) from 48 individuals of seven *Nucras* species (*N. boulengeri*, *N. holubi*, *N. intertexta*, *N. ornata*, *N. lalandii*, *N. tessellata*, and *N. livida*). Further data from Branch et al. (2019a) were downloaded from GenBank for additional sequences of *N. intertexta* ($n = 3$), *N. holubi* ($n = 1$), *N. taeoniolata* ($n = 2$), *N. tessellata* ($n =$

1), *N. livida* ($n = 1$), *N. lalandii* ($n = 2$), and *N. broadleyi* ($n = 2$), and for five outgroup taxa belonging to the southern African radiation of Eremiadinae (Engleder et al. 2013): *Australolacerta australis*, *Merolles knoxii*, *M. suborbitalis*, *Pedioplanis laticeps*, *P. namaquensis*, and *Heliobolus lugubris*. All of the sequences combined resulted in a final dataset of 73 individuals (see Table 2). Genomic DNA was extracted using the Qiagen DNAeasy Kit from whole tissues consisting of tail tips, liver, or skeletal muscle and stored in 95% ethanol. PCR amplification was performed on an Eppendorf Mastercycler gradient thermocycler using the primer pairs METF1 (5'-AAGCTTTCGGGCCCATACC-3') (Macey et al. 1997) and CO1R1 (5'-AGRGTG CCAATGTCTTTGTGRTT-3') (Arèvalo et al. 1994) for ND2, ND4F (5'-CACCTATGACTAC CAAAAGCTCATGTAGAAGC-3') and Leu (5'-CATTACTTTTACTTTGGATTTGCACCA-3') (Arèvalo et al. 1994) for ND4, and 16Sa-l (5'-CGCCTGTTTATCAAAAACAT-3') and 16S-H (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991) for 16S. PCR products were visualized using 1.5% agarose gels before being purified with the AMPure magnetic bead solution kit (Agencourt Bioscience, Beverly, Massachusetts, USA). Cycle sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and then samples were purified using the CleanSeq magnetic bead kit (Agencourt Bioscience, Beverly, Massachusetts, USA). Sequences were analyzed on an ABI 3730xl DNA analyzer and subsequently assessed in Geneious Prime v2020.0.5, where an initial sequence alignment was constructed using the MUSCLE v3.8.31 alignment tool and then manually adjusted by eye.

Phylogenetic analysis. Phylogenetic tree reconstruction used 1,686 base pairs (bp) derived from three mitochondrial markers (16S, ND2, and ND4). Variable and parsimony informative sites were summarized using the tool AMAS (Alignment Manipulation and Summary) (Borowiec 2016). Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed on the CIPRES Science Gateway v3.3 (Miller et al. 2010). Analyses were performed on each individual gene and on the concatenated set of mitochondrial genes. Each dataset was partitioned using PartitionFinder v2.1.1 (Lanfear et al. 2017), employing the partitioning schemes supported by the Bayesian Information Criterion (BIC) score for each analysis. The analysis resulted in three partitions for the concatenated mtDNA analysis: (1) the first codon position of ND2 and the third codon of ND4; (2) the second codon position of ND2, the first codon position of ND4, and the entire 16S gene; and (3) the third codon position of ND2 and the second codon position of ND4. All ML partitions were run under the GTR+ Γ model of evolution using RAxML v8.1.24 (Stamatakis 2014) for

Sandveld lizards (*Nucras*) of Namibia

Table 1. Measurements and scale counts from Namibian specimens of *Nucras damarana* and *N. aff. broadleyi*. Scale counts are provided for the right side of the body only and femoral pore counts from the left, except for MCZ R190201, which serves as the basis for the redescription of *Nucras damarana* provided in this paper.

	<i>N. damarana</i>	<i>N. damarana</i>	<i>N. damarana</i>	<i>N. damarana</i>	<i>N. damarana</i>	<i>N. aff. broadleyi</i>
	MCZ R190201	CAS 180421	CAS 193676	CAS 193807	CAS 193668	CAS 214642
SVL	57.6	41.6	31.8	42.6	38.8	58.2
TrW	9.4	6.5	3.8	6.7	5.5	9.7
TailL	107.0	101.0	67.5	89.0	95.0	120
TailW	5.0	3.3	2.2	3.3	3.4	4.7
AGL	32.0	20.8	14.7	24.7	20.0	31.5
HumL	5.0	4.7	3.2	4.6	4.4	5.8
ForeaL	5.5	4.8	3.7	3.9	3.8	5.5
FemL	7.7	6.9	5.5	5.5	5.3	7.5
CrusL	8.5	7.4	5.5	6.2	6.5	8.2
PesL	12.2	12.4	9.8	11.3	11.0	12.2
HeadL	11.6	11.4	8.4	10.6	9.9	13.1
HeadW	7.6	6.7	4.3	5.3	5.5	7.2
HeadD	5.5	4.6	3.1	4.3	4.2	5.5
CSn	17.8	15.2	11.6	13.4	14.5	19.1
OrbD	2.0	2.0	1.2	1.9	1.9	2.0
Neye	3.8	3.1	2.3	3.1	2.6	3.7
Eyee	4.5	3.9	2.4	3.6	3.8	5.0
EarH	2.2	1.9	1.3	1.7	1.7	2.1
EarW	1.3	0.8	0.5	1.1	0.9	1.3
Chin Shields	4/4	4	4	4	4	4
Femoral pores (per thigh)	12/12	13	13	13	13	13
Supralabials	8/8	7	7	7	7	6
Infralabials	8/8	6	7	6	6	6
Supraoculars	4/4	4	4	4	4	4
Supraciliaries	7/8	7	7	7	7	7
Supraciliary granules	5/5	6	4	6	6	6
Supratemporals	2/3	2	3	2	3	4
Dorsal scale rows at midbody	36	40	37	37	36	38
Ventral scale rows	8	8	8	8	8	8
Ventral scales in longitudinal series	33	28	28	28	29	34
Subdigital lamellae	9/9-14/13-18/18-26/25-?/14	7-13-18-25-12	8-13-18-24-14	9-13-18-24-14	10-13-17-24-14	10-13-18-23-13

Table 2. Samples used in molecular phylogenetic analysis with associated GenBank accession numbers. Specimen collection standard abbreviations are detailed in **Materials and Methods**.

GenBank accession number									
Taxon name	Specimen	Field/tissue #	Country	Province/Region	Specific locality	Latitude	Longitude	ND2	ND4
<i>Nucras aurantiacus</i>	NMB R11626	USHNS01	South Africa	Western Cape	Lamberts Bay	32°05′04.36″S	18°21′26.9″E	MH023414	—
<i>Nucras aurantiacus</i>	NMB R11627	USHNS02	South Africa	Western Cape	Lamberts Bay	32°05′04.36″S	18°21′26.9″E	MH023415	—
<i>Nucras boulengeri</i>	PEM R16773	KC 20	Tanzania	Mara	Klein’s Camp	01°49′16.2″S	35°14′30.5″E	MG846513	—
<i>Nucras</i> aff. <i>broadleyi</i>	CAS 214642	AMB 6405	Namibia	Kunene	59 km W of Kamanjab	19°39′14″S	14°21′03″E	MT137656	MT137653
<i>Nucras broadleyi</i>	PEM R24005	AG 018	Angola	Namibe	10 km W Lola	14°17′25″S	13°31′50″E	—	—
<i>Nucras broadleyi</i>	PEM R24157	AG 166	Angola	Namibe	8.8 km SW Farm Mucungo	14°48′06″S	12°25′09″E	—	MN265871
<i>Nucras damarana</i>	MCZ R190201	AMB 8037	Namibia	Omusati	60 km SE of Ruacana	17°29′8.1″S	14°51′57.9″E	MT137660	MT137655
<i>Nucras holubi</i>	—	MB 21672	South Africa	Limpopo	Farm Kalkfontein	24°54′47″S	30°04′17″E	MT137657	—
<i>Nucras holubi</i>	CAS 234138	MCZ F38793	South Africa	Limpopo	Farm Pylkop	22°45′52″S	29°44′28″E	MG846533	MG846580
<i>Nucras holubi</i>	MCZ R184459	AMB 8313	South Africa	Limpopo	Farm Celine	22°41′29″S	29°31′42″E	MG846532	MG846563
<i>Nucras holubi</i>	NMB R11613	MBUR 01001	South Africa	Limpopo	Greater Kuduland Safaris	22°38′28″S	30°18′50″E	MG846534	MG846577
<i>Nucras holubi</i>	NMB R11615	MBUR 01027	South Africa	Limpopo	Greater Kuduland Safaris	22°38′28″S	30°18′50″E	MG846515	MG846578
<i>Nucras holubi</i>	PEM R17430	—	South Africa	KwaZulu-Natal	Mkhuze Game Reserve	27°37′17″S	32°10′40″E	MT137661	—
<i>Nucras holubi</i>	PEM R18647	RSP420	South Africa	Limpopo	Venetia Limpopo Reserve	22°19′08″S	29°20′53″E	—	HG005187
<i>Nucras holubi</i>	PEM R22813	MBUR 00232	South Africa	Limpopo	Bochum	23°16′52″S	29°08′10″E	MT137658	—
<i>Nucras holubi</i>	PEM R22814	MBUR 00260	South Africa	Limpopo	Bochum	23°16′52″S	29°08′10″E	MT137659	MT137654
<i>Nucras intertexta</i>	—	LMH 000095	South Africa	Limpopo	Bergplaats Game Lodge	24°00′1.9″S	29°58′56.6″E	MG846550	MG846564
<i>Nucras intertexta</i>	—	MB 20952	South Africa	Northern Cape	Farm Blackridge	28°49′02″S	22°32′42″E	MG846542	MG846568
<i>Nucras intertexta</i>	—	MBUR 21183	South Africa	Northern Cape	Farm Lemoenfontein	30°51′10″S	23°37′36″ E	—	HG005194
									HG005222

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Table 2 continued. Samples used in molecular phylogenetic analysis with associated GenBank accession numbers. Specimen collection standard abbreviations are detailed in **Materials and Methods**.

Taxon name	Specimen	Field/tissue #	Country	Province/Region	Specific locality	Latitude	Longitude	GenBank accession number			
								ND2	16S rRNA	ND4	ND4
<i>Nucras intertexta</i>	CAS 234193	MCZ F38851	South Africa	Limpopo	Lajuma Wilderness Camp	23°02'21"S	29°26'59"E	MG846541	MG846602	MG846581	
<i>Nucras intertexta</i>	CAS 234212	MCZ F38872	South Africa	Limpopo	Lajuma	23°02'10"S	29°25'41"E	MG846540	—	—	—
<i>Nucras intertexta</i>	PEM R18257	RSP030	South Africa	Northern Cape	Rooipoort	28°36'45"S	24°08'41"E	—	HG005191	HG005219	
<i>Nucras intertexta</i>	PEM R18661	RSP 277	South Africa	Northern Cape	Tswalu	27°17'52"S	22°27'14"E	—	HG005190	HG005218	
<i>Nucras intertexta</i>	PEM R20958	MB 21288	South Africa	Northern Cape	Farm Mansfield	27°41'28"S	23°25'00"E	MG846525	—	—	—
<i>Nucras intertexta</i>	PEM R21065	MB 21406	South Africa	North West	About 1 km SE of Majankeng	27°07'04"S	23°57'31"E	MG846543	—	—	—
<i>Nucras lalandii</i>	—	MB 20982	South Africa	Western Cape	11 km ESE Herbertsdale	34°02'49"S	21°53'07"E	—	HG005197	HG005225	
<i>Nucras lalandii</i>	—	MBUR 00483	South Africa	Eastern Cape	Farm Bamboesberg	31°36'52"S	26°18'50"E	—	HG005196	HG005224	
<i>Nucras lalandii</i>	NMB R10904	MBUR 00484	South Africa	Eastern Cape	Bamboesberg, Farm Bamboeshoek	31°36'52"S	26°18'50"E	MG846549	MG846591	MG846572	
<i>Nucras lalandii</i>	PEM R21025	MBUR 00414	South Africa	Eastern Cape	1 km S of Siphepheto village	30°33'03"S	28°50'22"E	MG846548	MG846590	MG846571	
<i>Nucras lalandii</i>	PEM R21026	MBUR 00415	South Africa	Eastern Cape	1 km S of Siphepheto village	30°33'03"S	28°50'22"E	MG846544	—	—	—
<i>Nucras lalandii</i>	PEM R22815	MBUR 00411	South Africa	Eastern Cape	1 km W of Siphepheto village	30°32'08"S	28°49'38"E	MG846547	MG846589	MG846570	
<i>Nucras lalandii</i>	PEM R22816	MBUR 00412	South Africa	Eastern Cape	1 km W of Siphepheto village	30°32'08"S	28°49'38"E	MG846546	—	—	—
<i>Nucras lalandii</i>	PEM R22817	MBUR 00432	South Africa	Eastern Cape	Near Fever village	30°32'07"S	28°49'37"E	MG846545	—	—	—
<i>Nucras lalandii</i>	PEM R22818	MBUR 00485	South Africa	Eastern Cape	Bamboesberg, Farm Bamboeshoek	31°36'52"S	26°18'50"E	MG846516	MG846592	MG846573	
<i>Nucras lalandii</i>	PEM R22819	MBUR 00550	South Africa	Eastern Cape	1.5 km E of Toisekraal	31°49'45"S	26°45'36"E	MG846560	MG846593	MG846574	

Table 2 continued. Samples used in molecular phylogenetic analysis with associated GenBank accession numbers. Specimen collection standard abbreviations are detailed in **Materials and Methods**.

GenBank accession number									
Taxon name	Specimen	Field/tissue #	Country	Province/Region	Specific locality	Latitude	Longitude	ND2	ND4
<i>Nucras lalandii</i>	PEM R9707	—	South Africa	Eastern Cape	Mtentu River nr. Holy Cross Mission	31°09'19"S	29°44'44"E	MG846517	—
<i>Nucras livida</i>	PEM R18747	KTH 08-071	South Africa	Western Cape	Little Karoo	33°32'50"S	21°12'55"E	—	HG005227
<i>Nucras livida</i>	PEM R19087	MB 21209	South Africa	Northern Cape	Farm Kareehoek	30°10'37"S	23°28'54"E	MG846535	MG846569
<i>Nucras livida</i>	PEM R19094	MB 21216	South Africa	Northern Cape	~44 km SW of Strydenburg	30°08'27"S	23°15'31"E	MG846536	—
<i>Nucras livida</i>	PEM R19103	MB 21225	South Africa	Northern Cape	Farm Goodhope	30°07'28"S	23°18'48"E	MG846519	—
<i>Nucras livida</i>	PEM R19108	MB 21230	South Africa	Northern Cape	Farm Kareehoek	30°10'37"S	23°28'54"E	MG846537	—
<i>Nucras livida</i>	PEM R19116	MB 21238	South Africa	Northern Cape	Farm Goodhope	30°07'28"S	23°18'48"E	MG846538	—
<i>Nucras livida</i>	PEM R22820	MBUR 00632	South Africa	Eastern Cape	Farm Suurhoek	32°51'37"S	24°27'25"E	MG846520	MG846575
<i>Nucras livida</i>	PEM R22821	MBUR 00646	South Africa	Eastern Cape	Farm Matjiesfontein	32°50'50"S	24°25'12"E	MG846521	—
<i>Nucras livida</i>	PEM R22822	MBUR 00670	South Africa	Eastern Cape	Farm Matjiesfontein	32°50'06"S	24°26'28"E	MG846539	—
<i>Nucras livida</i>	PEM R22823	MBUR 00687	South Africa	Western Cape	Farm Tierberg	33°09'12"S	22°15'56"E	MG846518	MG846576
<i>Nucras ornata</i>	NMB R10657	MBUR 01169	South Africa	KwaZulu-Natal	Manyiseni region	26°54'55"S	32°03'04"E	MG846529	—
<i>Nucras ornata</i>	NMB R10658	MBUR 01230	South Africa	KwaZulu-Natal	Manyiseni region	26°54'55"S	32°03'04"E	MG846527	—
<i>Nucras ornata</i>	NMB R10659	MBUR 01251	South Africa	KwaZulu-Natal	Usutu Gorge	26°51'58"S	32°09'17"E	MG846551	—
<i>Nucras ornata</i>	NMB R10660	MBUR 01262	South Africa	KwaZulu-Natal	1.5 km SE of Ekuhleleni	26°51'38"S	32°03'08"E	MG846552	—
<i>Nucras ornata</i>	PEM R17591	AMB 8635	South Africa	Mpumalanga	N of Swaziland border	25°05'27"S	31°59'23"E	MG846526	—
<i>Nucras ornata</i>	PEM R5906	—	South Africa	KwaZulu-Natal	Manjiseni region	27°04'45"S	32°02'25"E	MG846531	—
<i>Nucras taeniolata</i>	—	HZ251	South Africa	Eastern Cape	—	—	—	HG005208	HG005230
<i>Nucras taeniolata</i>	PEM R18080	—	South Africa	Eastern Cape	Nyati Camp	32°59'03"S	26°50'01"E	—	HG005210

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Table 2 continued. Samples used in molecular phylogenetic analysis with associated GenBank accession numbers. Specimen collection standard abbreviations are detailed in **Materials and Methods**.

Taxon name	Specimen	Field/tissue #	Country	Province/Region	Specific locality	Latitude	Longitude	GenBank accession number			
								ND2	16S rRNA	ND4	
<i>Nucras tessellata</i>	—	MB 20724	South Africa	Northern Cape	Farm Donkiedam	30°55'27"S	19°02'51"E	MG846557	MG846586	MG846567	
<i>Nucras tessellata</i>	—	RBH 3468	South Africa	Northern Cape	Kgalagadi Transfrontier Park	26°24'12.1"S	20°41'1.46"E	MG846524	MG846604	—	
<i>Nucras tessellata</i>	CAS 201917	AMB 5070	South Africa	Northern Cape	Richtersveld National Park	28°6'40"S	17°1'10"E	MG846522	—	MG846561	
<i>Nucras tessellata</i>	CAS 206723	AMB 5582	South Africa	Northern Cape	Groenriviermond	30°51'29"S	17°34'43"E	MG846553	—	MG846562	
<i>Nucras tessellata</i>	CAS 206725	AMB 5584	South Africa	Northern Cape	Groenriviermond	30°51'29"S	17°34'43"E	MG846555	—	—	
<i>Nucras tessellata</i>	NMB R10781	MBUR 21061	South Africa	Northern Cape	Farm Good Hope	29°52'04"S	22°33'22"E	MG846559	—	—	
<i>Nucras tessellata</i>	NMB R11497	MBUR 1393	South Africa	Northern Cape	Farm Leeuberggrivier	30°27'48"S	19°26'52"E	MG846523	MG846598	—	
<i>Nucras tessellata</i>	NMB R11514	MBUR 1394	South Africa	Northern Cape	Farm Narosies	30°33'03"S	19°34'53"E	MG846558	MG846599	MG846579	
<i>Nucras tessellata</i>	PEM R16872	MB 20687	South Africa	Northern Cape	Farm Kamas	30°35'28"S	18 49 20 E	—	HF951557	HF951536	
<i>Nucras tessellata</i>	PEM R16873	MB 20650	South Africa	Northern Cape	Farm Loerkop	30°39'23"S	19°20'33"E	MG846554	MG846584	MG846565	
<i>Australolacerta australis</i>	—	MH 0531	South Africa	Western Cape	Buffelshoek Corner	33°28'19"S	19°23'41"E	DQ871094	DQ871152	—	
<i>Heliobolus lugubris</i>	CAS 234139 MCZ	MCZ F37894	South Africa	Limpopo	Farm Pylkop	22°45'52"S	29°44'28"E	DQ871084	DQ871142	—	
<i>Heliobolus lugubris</i>	R184277	MCZ F37870	Namibia	Kunene	Kamanjab Rest Camp	19°37'47"S	14°48'56"E	DQ871083	DQ871141	—	
<i>Meroles knoxii</i>	CAS 206782	AMB 5629	South Africa	Northern Cape	Port Nolloth	29°15'11.4"S	16°52'40.8"E	DQ871088	DQ871146	—	
<i>Meroles suborbitalis</i>	PEM R16978	MB 20696	South Africa	Northern Cape	Farm Kamans	30°35'17"S	18°50'49"E	MG846556	MG846585	MG846566	
<i>Pedioplanis laticeps</i>	PEM R17212	JSM 018	South Africa	Northern Cape	Bezantsgat Farm	32°29'34"S	19°35'34"E	DQ871067	DQ871125	—	
<i>Pedioplanis namaquensis</i>	CAS 200033	AMB 4558	South Africa	Northern Cape	vic. Kodas Ruins	28°14'58"S	16°56'41"E	DQ871043	DQ871101	—	

1,000 rapid nonparametric bootstrap replicates (BS), with values greater than 70% considered to be indicative of support. The BI PartitionFinder analysis resulted in the same partitioning scheme recovered for the ML analysis (Partitions 1–3 listed above) and were run using the GTR+I+ Γ (Partitions 1 and 2) and HKY+ Γ (Partition 3) models of evolution. MrBayes v3.2.7 (Ronquist et al. 2012) was used to perform the BI analysis, and it was run for 50,000,000 generations sampling every 10,000 generations. Convergence of the Markov chains was assessed by eye using Tracer v1.6 (Rambaut et al. 2014) and the initial 25% of trees were discarded as burn-in. Posterior probabilities (PP) greater than 0.95 were considered to be indicative of support.

Results

Molecular phylogenetics. Final alignments for the three mitochondrial markers were as follows: ND2, 403 bp (226 variable, 193 parsimony informative); ND4, 732 bp (329 variable, 278 parsimony informative); and 16S, 551 bp (157 variable, 123 parsimony informative). There were no conflicts in the tree topologies between the BI and ML analyses and both analyses retrieved generally high nodal support, with some notable exceptions, throughout their respective trees (Fig. 1). *Nucras boulengeri* and *N. broadleyi* (including our *Nucras* sp. from northern Namibia) form a clade sister to all other *Nucras* species (BS = 89%, PP = 0.92). Among the remaining named taxa there are two major clades (although this split received weak nodal support; BS = 46%, PP = 0.58): one which includes *N. holubi* (BS = 100%; PP = 1.0), and sister taxa *N. intertexta* and *N. ornata* (BS = 100%, PP = 0.99), and another which is comprised of all remaining *Nucras* species. The latter includes *N. aurantiacus*, which is sister (BS = 99%, PP = 1.0) to a clade containing *N. livida* which is itself sister (BS = 99%, PP = 1.0) to a clade (BS = 95%, PP = 0.99) containing *N. tessellata* and *N. taeniolata*, with *N. taeniolata* appearing nested within the broader *N. tessellata* clade (BS = 90%, PP = 0.92). Pairwise uncorrected ND4 distances between *N. taeniolata* and sister *N. tessellata* samples (CAS 201917, 206723) were 0.45–6.08% (mean 3.26%).

Within *N. holubi* there appear to be three highly divergent clades. The first is comprised of individuals from the Limpopo and KwaZulu-Natal provinces of South Africa (BS = 100%, PP = 1.0), which is sister (BS = 100%, PP = 1.0) to a second clade comprised of an individual from Namibia, which is sister (BS = 80%, PP = 0.89) to another set of specimens collected from Limpopo, South Africa—thus rendering the South African *N. holubi* paraphyletic with respect to the single specimen collected in northern Namibia (MCZ R190201). Pairwise uncorrected ND2 distances from the Namibian *N. holubi* were 12.30–12.84% (mean 12.50%) to their sister South African *N. holubi*, and 14.25–14.56% (mean 14.40%) to the remaining (outgroup) South African *N.*

holubi. For 16S, there was a 19.14% mean difference between the Namibian specimen and its sister clade of *N. holubi*, a 12.83% difference from the other South African *N. holubi*, and a 19.66% mean divergence between the two South African *N. holubi* clades. Comparable values for ND4 were 13.1%, 17.84%, and 15.58%. The mean intraclade divergences for the sister clade to the Namibian sample was 2.01% for ND2 (range 0.26–3.42%). Divergences were 2.85% for ND4 and 1.09% for 16S for the only two samples available for these markers. Pairwise uncorrected distances between the Angolan *N. broadleyi* and its Namibian sister were 15.03–15.69% and 8.24% for 16S and ND4, respectively, and there was a 7.83% 16S difference between the two Angolan samples (noting that no ND2 data were available for the Angolan samples and ND4 was available only for PEM R24157).

Morphology

***Nucras damarana*.** The Namibian specimen genotyped as a member of the *N. holubi* clade (MCZ R190201) was compared to specimens of *N. holubi* from Limpopo Province, South Africa, from which the original syntype series was derived (Limpopo Valley, Transvaal; Steindachner 1882). It was also compared to the type material of *N. intertexta damarana* (Fig. 2). Parker (1936) distinguished *N. i. damarana* from its congeners by its small size (maximum 54 mm SVL) and the shortening of the interparietal such that the parietals form a suture behind it. Broadley (1972) recorded 24 specimens of *Nucras taeniolata ornata* from Southwest Africa [=Namibia], all of which may be assumed to be the same taxon described by Parker (1936). He further demonstrated that one of Parker's distinguishing features for *N. i. damarana*, the contact of the parietals separating the interparietal from the occipital, was not constant among the Namibian material and that it also occurred in other congeners.

However, Broadley (1972) did find that his Namibian “*ornata*” were smaller than all other members of the *N. tessellata* group, except for *N. taeniolata ornata* (today *N. holubi*) from Gaborone, Botswana, with no individuals larger than 57 mm SVL. He also demonstrated that the Namibian specimens had the lowest mean number of transverse scale rows at midbody (39), with almost no overlap between this group and his other populations of *N. ornata* (= *N. ornata* + *N. holubi*). This group also exhibited one of the lowest total numbers of femoral pores (lowest minimum number of 21, and second only in mean to the Kimberley District specimens [of *N. holubi*] of < 26).

MCZ R190201 (Figs. 3, 4A) is only slightly larger than the largest specimen recorded by Broadley (1972: 57.6 vs. 57 mm SVL), and has only 36 midbody scale rows and a total of only 24 femoral pores. Four additional specimens of Namibian “*N. holubi*” examined here

Sandveld lizards (*Nucras*) of Namibia

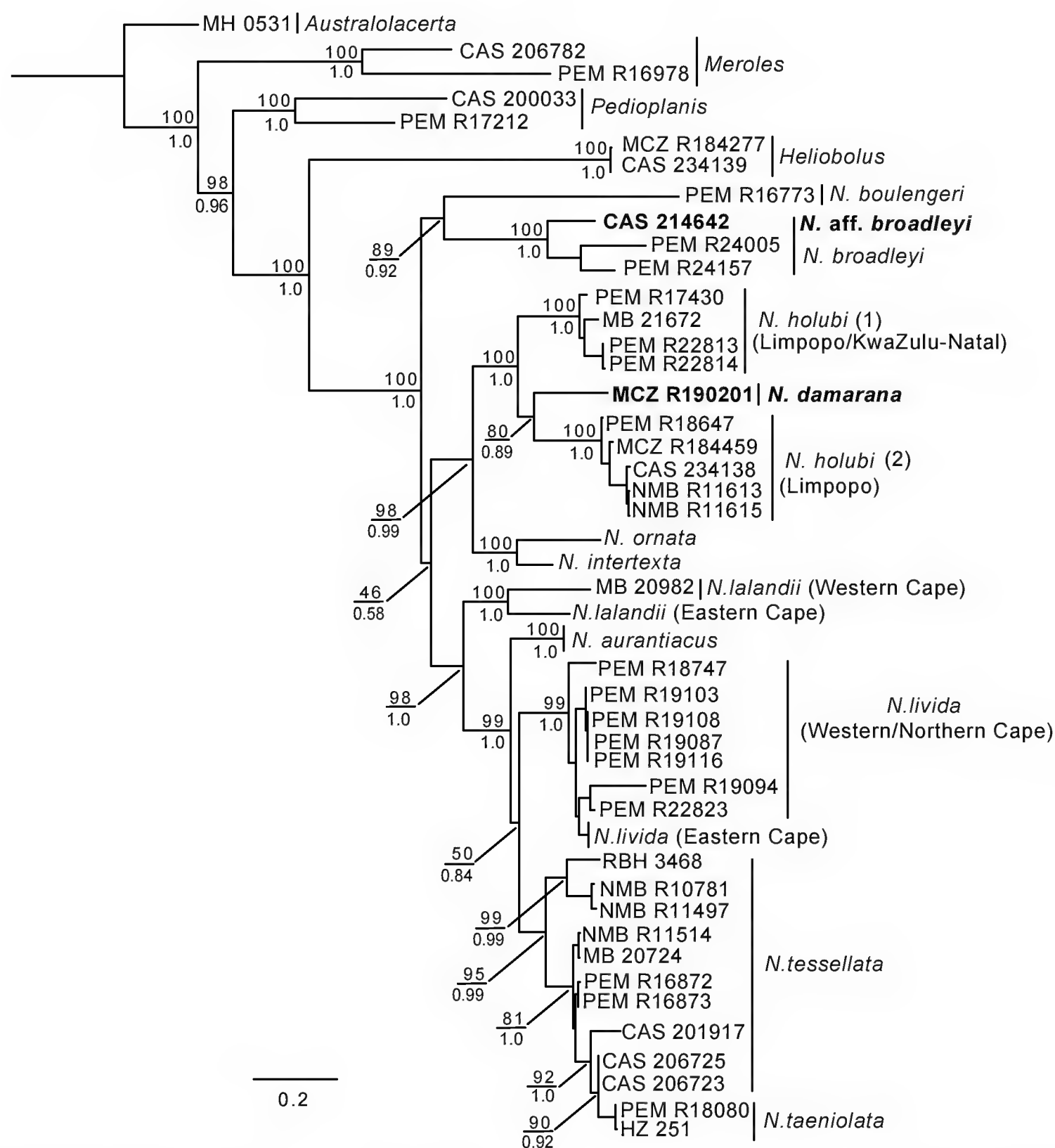


Fig. 1. Concatenated mitochondrial RaxML phylogram of the genus *Nucras* and outgroups used in this study. Bootstrap support (above) and posterior probabilities (below) are shown for the species level and more inclusive nodes. Select nodes containing individuals with identical or minor sequence divergence (less than 2%) from shared localities and from non-focal taxa have been collapsed in order to condense overall tree size; but a full list of the specimens used in this study can be found in Table 1.

(CAS 180421, 193807, 193668, 193676) are likewise consistent, with maximum counts of 40 midbody scale rows and 26 femoral pores. Further, Parker's (1936) statement that the occipital scale was reduced in his new taxon is also borne out by our specimens, two of which have a small occipital scale and three of which have no distinct occipital scale (Fig. 3C). These features separate the disjunct Namibian "*N. holubi*" from the southeastern African *N. holubi sensu stricto* and, along with the large genetic divergence and geographic disjunction between them, support the resurrection of *Nucras damarana* Parker, 1936 as a distinct taxon that is apparently endemic to northern Namibia.

Parker's (1936) description of *Nucras intertexta damarana* was written at a time of great confusion over species boundaries within *Nucras*. Indeed, despite its obvious similarity to *N. holubi* and *N. ornata*, his

comparisons were chiefly with *N. intertexta*. This confusion still existed through the revisionary work of Broadley (1972), who synonymized several taxa now considered distinct from one another. Parker's (1936) diagnosis was brief and is now inadequate to unambiguously distinguish the taxon among all of its congeners. As a consequence, we provide a new diagnosis for the taxon and a detailed description of MCZ R190201.

Nucras damarana Parker, 1936

Nucras tessellata [part]: Werner (1910: 329).

Nucras intertexta damarana Parker, 1936: 135.

Syntypes: NHMUK 1946.8.6.17–24 [originally 1936.8.1.534–541]. Sessekab [=Sissekab], N.W. of Otavi, 1,300 m. Coll. Karl Jordan, 10–12 November 1933.



Fig. 2. Syntype series of *Nucras intertexta damarana* Parker, 1936 (NHMUK 1946. 1946.8.6.17–24) from Sissekab (-19.328645, 17.196238), Otjozondjupa Region, Namibia. The image has been modified to provide a uniform background and some extraneous string has been deleted. Photo by A.M. Bauer.

Nucras intertexta holubi [part]: FitzSimons (1943: 320).
Nucras taeniolata ornata [part]: Broadley (1972: 13).
Nucras taeniolata holubi [part]: Jacobsen (1989: 453).
Nucras holubi: Bates (1996: 35) [by implication]; Branch (1998: 169) [explicit for Namibian populations].

Referred material

Kunene Region: Otjivakundu (-17.120881, 13.258644) DNMNH 38806–07; 25 km N of Etengua (-17.292402, 12.945191) DNMNH 49033; 15 km N of Opuwo (-17.944228, 13.857124) DNMNH 48964–65; Opuwo (-18.061608, 13.83867) NMW 35352, DNMNH 24479, DNMNH 32352 [given as DNMNH 32351 by Broadley (1972)], DNMNH 33021, DNMNH 38911–14, DNMNH 71317; 57.9 km SW of Opuwo on Opuwo-Orupembe Rd. (-18.25533, 13.50200) CAS 193807; Okamangudona (-18.264261, 13.513046) DNMNH 71284; Hoarusib River, 92 km S of Opuwo (-18.269277, 13.216553) DNMNH 51219; Kaoko Otavi (-18.299656, 13.654006) SAM ZR-017494; Otjitundua (-18.65000, 14.23333) SAM ZR-017535; 35 km W of

Kamanjab on Kamanjab-Torrabaai Rd. (-19.57617, 14.59877) CAS 193668; Torrabaai Rd., 48.6 km W of Farm Franken entrance (-19.63633, 14.38267) CAS 180421; 59.3 km W of Kamanjab on Kamanjab-Torrabaai Rd. (-19.65167, 14.35558) CAS 193676; Outjo (-20.116667, 16.15000) SAM ZR-017507; Farm Kaokokroou [487] (-20.35948, 14.905159) NMW 31109. **Omusati Region:** 60 km SE of Ruacana crossroads (-17.48558, 14.86608) MCZ R190201; Ombombo (-17.940000, 14.310000) SAM ZR-017519. **Oshana Region:** Emono, 2 km SW of Onayena (-17.775004, 15.678522) NMB R07448; Oshakati (-17.785131, 15.698611) DNMNH 38613, DNMNH 45761. **Oshikoto Region:** Namutoni (-18.807768, 16.940231) ZFMK 18579–80; Chudop Waterhole (-18.856125, 16.923499) NMNW 5482; DNMNH 57024–25; Halali, Etosha National Park (-19.033333, 16.466667) DNMNH 56393. **Otjozondjupa Region:** Sissekab (-19.328645, 17.196238) NHMUK 1946.8.6.17–24; Kombat (-19.713265, 17.710345) DNMNH 30464. **Note:** Broadley (1972) listed DNMNH 22225 from Stamprietfontein in the Hardap



Fig. 3. *Nucras damarana* (MCZ R-190201). (A) Whole body dorsum, (B) lateral view of head, and (C) dorsal view of head. Note the single row of spots and dashes on each flank, and the absence of a discrete occipital scale. Photos by M. Murdoch.

Region (-24.33333, 18.40000), however, this specimen is listed in the DNMNH database as being from Opuwo, Kunene Region. Given the large geographic disjunction between Stamprietfontein and all other localities and the fact that there is another anomalous specimen record from Stamprietfontein in the Ditsong collection (*Causus rhombeatus*; DNMNH 22222), we consider the record as dubious and have omitted it.

Diagnosis. A small *Nucras* (maximum 57.6 mm SVL) with eight longitudinal series of enlarged ventral plates, a series of small granules between the supraoculars and supraciliaries, occipital scale reduced or absent, and enlarged plates on the underside of the forearm. Adult dorsal color pattern characterized by three distinct pale longitudinal stripes extending from the nape to the tail base, an additional pale stripe at ventrolateral margin of flanks, flank markings comprise spots or horizontal dashes typically in a single line, and tail not brightly colored (Figs. 3A, 4A).

The new species may be distinguished from *N. lalandii* by the presence of enlarged plates under the forearms and from *N. lalandii* and *N. boulengeri* by the presence of a series of small granules between the supraoculars and supraciliaries. In its color pattern (see above), it is distinct from *N. aurantiaca* (no dorsal markings), *N. scalaris* (dark crossbands), *N. lalandii* (dark blotches or ocelli or both, forming transverse bands, but never stripes), *N. intertexta* (pale dorsal spots and/or irregular thin crossbands or reticulations), *N. livida* (six stripes on nape), *N. tesellata* (two or four stripes on nape, stripes generally not extending to sacrum; tail and hindbody reddish), *N. broadleyi* (usually four stripes on nape, see section on this taxon below), *N. taeniolata* (8–11 stripes on nape), *N. caesicaudata* (5–7 pale dorsal stripes; tail pale blue), and *Nucras ornata*

(vertebral line often lacking; markings on flanks oriented vertically; tail reddish). *Nucras damarana* is most similar to *N. holubi*, with which it has long been confused. Both taxa share three prominent pale stripes and other basic pattern elements. However, the former species is much smaller, reaching only 57.6 mm SVL, whereas true *N. holubi* may reach 73 mm SVL (Jacobsen 1989), and has a lower number of midbody dorsal scale rows (34–42 (36–40 in our sample of five)) versus 41–65 in *N. holubi* in the former Transvaal (Jacobsen 1989) and 44–58 in Botswana (Broadley 1972). In addition, the vertebral stripe in *N. damarana* does not typically have crisply demarcated edges (Figs. 3A, 4A; vs. cleanly demarcated by a dark brown border in *N. holubi*, Fig. 4B) and flank markings in *N. damarana* generally form a single line of irregularly shaped pale spots, sometimes anteriorly the spots are connected (vs. frequently forming two or rarely more lines of irregularly shaped pale spots in *N. holubi*).

Description of MCZ R190201 (field number AMB 8037). Measurements are given in Table 1. Body moderately slender and elongate (AGL/SVL 0.56), trunk longer than hind limbs (AGL/[FemL + CrusL + PesL] 1.11), tail longer than SVL (TailL/SVL 1.86), moderately slender and tapering. Limbs short, pes longer than shank or femur (PesL/FemL 1.62; PesL/CrusL 1.47). Head moderately large (HeadL/SVL 0.20), distinct from neck, slightly elongate (HeadW/HeadL 0.66), not depressed (HeadD/HeadL 0.47). Snout blunt, short (NEye/HeadL 0.33), almost twice eye diameter (NEye/OrbD 1.9). Eye relatively large (OrbD/HeadL 0.17); lower eyelid scaly, with four large translucent/semi-opaque scales surrounded by a rim of small granules. Eye to ear distance more than 1.5 times diameter of eye (EyeE/OrbD 2.25).

Ear opening vertical, much taller than wide (EarH/EarW 1.62), without projecting lobules, bordered

posteriorly by a series of tiny granules and anteriorly by a series of slightly larger, elongate scales and anterior to this a vertical row of enlarged juxtaposed scales; tympanic shield narrow, three times the size of cheek scales. Rostral approximately as wide as deep, strongly gabled, separating supranasals for most of their length; loreal region flat to very slightly concave. Supralabials 8R/8L; increasing in size posteriorly to the sixth right, fifth left, which is largest; in subocular position and possessing a strong longitudinal furrow running the length of the scale in continuation with the contact border between the anteriormost supralabial and loreal. Infralabials 8R/8L, all much longer than wide.

Nostrils semicircular, surrounded by enlarged supranasal, and two postnasals, each approximately one-fourth the size of supranasal. Two loreal scales; anterior loreal trapezoidal, bordered anteriorly by two postnasals, dorsally by frontal and prefrontal, ventrally by second supralabial (fully) and supralabials one and three (point contact only); posterior loreal five-sided, posterior face much taller than anterior, twice as long as anterior loreal, bordered dorsally by prefrontal, posteriorly by first (point contact only) supraocular, first supraciliary, three preocular scales, and ventrally by supralabials three and four. Supranasals in narrow contact with one another posteromedially; frontonasal roughly hexagonal, wider than long, with lateral apices projecting posteriorly, gabled anteriorly; prefrontals in broad contact with one another. Frontal scale approximately two times wider anteriorly than posteriorly, lateral terminus of frontal-frontoparietal suture lies posterior to border between

second and third supraoculars. Four supraocular scales on both sides, second and third much larger than first and fourth, 7R/8L supraciliary scales, smallest at midorbit, where there is a row of five supraciliary granules separating supraorbitals from main row of supraciliaries on each side. Parietals five-sided, much longer than wide, with slight forward projection wedged between frontoparietal and fourth supraciliary. Interparietal scale narrow and elongate, tapering to a point posteriorly, separating posteriormost portion of frontoparietals from one another and completely separating left and right parietals; parietal window small but distinct; no clear occipital scale present (Fig. 3C). 2R/3L supratemporal scales, anterior narrow and elongate, posterior less than half the size of anterior, but much larger than scales of cheek region.

Mental roughly semicircular, broader than deep, roughly same width as rostral, bordered posteriorly by a pair of small chin shields in midline contact with one another and bordering first and second infralabials. Second set of chin shields larger and also in contact with each other medially, extending dorsolaterally to the margin of the jaw, resulting in a loss of contact between second and third infralabials (condition not seen in other specimens; Fig. 3B). Third pair larger still and also in contact with each other medially, bordering third and fourth infralabials. Fourth pair of chin shield pairs 1.5 times as large as third and widely separated from one another medially, bordering infralabials 4–7. Indistinct gular fold present, scales anterior to this roughly hexagonal and becoming longitudinally elongate and



Fig. 4. (A) Life photo of *Nucras damarana* (MCZ R-190201) from 60 km SE of Ruacana crossroads (-17.48558, 14.86608), Omusati Region, Namibia. (B) Life photo of *Nucras holubi* from Alldays, Limpopo Province, Republic of South Africa. Note differences in dorsal and lateral patterning. Photos copyright by Johan Marais.

angled medially at approximately the level of the angle of the jaws; scales between gular fold and collar enlarged and rectangular. Twenty-seven scales between chin and collar; collar border comprising a series of eight enlarged scales, the largest in median position and rhomboidal in shape, decreasing in size dorsolaterally and anteriorly.

Dorsal pholidosis homogeneous, 36 longitudinal rows of small granules at midbody, becoming slightly larger and more flattened on flanks. Eight longitudinal rows of transversely widened ventral plates plus, lateralmost considerably smaller than the rest. Thirty-three transverse rows of ventral plates between axilla and groin.

Femoral pores extending to knee, 12 on each thigh, with left and right series separated by a diastema of two scales of roughly equal size. Scales in row immediately posterior to femoral pore row oval, approximately half the size of pore-bearing scales. Scales of rows anterior to pores much larger, one (distal) to three (middle and proximal) rows between pore-bearing scales and enlarged preaxial plates. Large, roughly semicircular patch of precloacal plates anterior to cloaca, constituent scales extremely large, largest bordering posterior margin medially, bordered laterally by one plate on each side, each one-sixth size of median plate, and anteriorly by two scales, each half the size of median plate, a semi-circular series of much smaller scales bordering the precloacal plates laterally and anteriorly.

Preaxial surface of forelimb covered with a series of transversely enlarged scales; postaxial surface covered by smaller, flattened juxtaposed scales. Scales on palms small, flattened, juxtaposed to subimbricate. Manual digits 4>3>5>2>1, all clawed. Preaxial aspect of thigh with large transverse plates, continuing on to shank and dorsum of pes, postaxial aspect with small, smooth, subimbricate scales, granular on shank. Scales on the sole small, smooth, granular to slightly elongate. Digits of pes 4>3>5>2>1, all clawed, bearing a series of smooth narrow subdigital lamellae, lamellar formulae: (L) 9-14-18-26-[5th toe missing], (R) 9-13-18-25-14.

Tail original, 107 mm in length, 22 elongate rectangular scales per whorl at level of knee of adpressed hindlimb. One row of dorsal scales for each ventral row. Basal portion of tail with most scales smooth and only scattered keeled scales, rapidly transitioning to keeled dorsal scales, and most of the tail with all scales keeled.

Color in alcohol (Fig. 3). Dorsum medium brown with three cream-colored longitudinal stripes, vertebral stripe narrower than lateral stripes and with less well-defined edges. Lateral stripes carry forward to the lateral edges of the parietal scales and fade out on the posterior frontal, adjacent to the posterior supraocular scales. Median stripe continuous with an irregular whitish marking centered on the interparietal scale. Flanks dark brown, bordered above by dorsolateral cream stripe and below by a thicker cream-colored stripe that is confluent with the white of the lateral surface of the neck and extends to the hindlimb insertion.

This stripe is bordered ventrally by a brown line that begins inconspicuously behind the axilla and widens posteriorly to include the lowest 2–3 rows of dorsal scales and the edges of the lateralmost ventral plates, terminating at the hindlimb insertion. Dark area of the flanks encompassing a discontinuous longitudinal series of white dashes and spots extending from the temporal region to the posterior edge of the hindlimb insertion.

Anterior portion of head densely speckled with brown pigment, fading on the ventral portion of the rostral and supralabials; infralabials without dark pigmentation; rims of eyelids with a narrow dark brown margin. Posterior supraoculars dark brown, this coloration continuing posteriorly along the dorsolateral margins of the head and confluent with the dark surfaces of the flanks. Forelimbs a mottled pale brown with darker posterior portions to most scales and a series of diffuse whitish spots. Hindlimbs with an irregular dark brown line running from the limb insertion onto the postaxial surface of the foot; another such line on the preaxial surface of the limb, passing over the front of the knee and terminating at the flexure of the ankle. The area between these lines occupied by a series of partly joined white spots with diffuse margins. The central cream stripe of the dorsum fades out on pygal portion of tail, but the lateral stripes widen and fuse posterior to this, yielding a more-or-less uniform pale brown dorsum of the tail. The darker coloration of the flanks continues as a diffuse stripe on the lateral surfaces of the proximal half of the tail before fading entirely. All ventral surfaces immaculate cream.

Color in life (Fig. 4A). Pattern as above. Dorsal ground color dark brown. Middorsal stripe an orange-brown, dorsolateral stripes off-white, ventrolateral stripes cream, spots on flanks a pale yellowish-cream, with diffuse orange-brown markings between some spots. Venter immaculate white.

Distribution. *Nucras damarana* has a broad distribution across northwestern Namibia, including the Kunene Region (exclusive of the Namib and pro-Namib in the west), as well as the Omusati, Oshana, and Oshikoto Regions and in the northwest of the Otjozondjupa Region (Fig. 5). Although unsupported by vouchers, it is likely to be present in the Ohangwena Region and into the southern Angolan Cunene Province.

Natural history. Parker (1936) recorded *Nucras damarana* only from Sessekab, which he described as “open forest.” The species occurs in several of the major landscape divisions of Namibia, including the Kalahari Sandveld, Kunene Hills, Cuvelai System, Central-western Plains, and Kamanjab Plateau, and spans a broad annual precipitation gradient from 150 mm in the west to approximately 500 mm in the east (Goudie and Viles 2015). Its distribution falls entirely within the Tree and Shrub Savanna biome, with the majority of localities within areas characterized

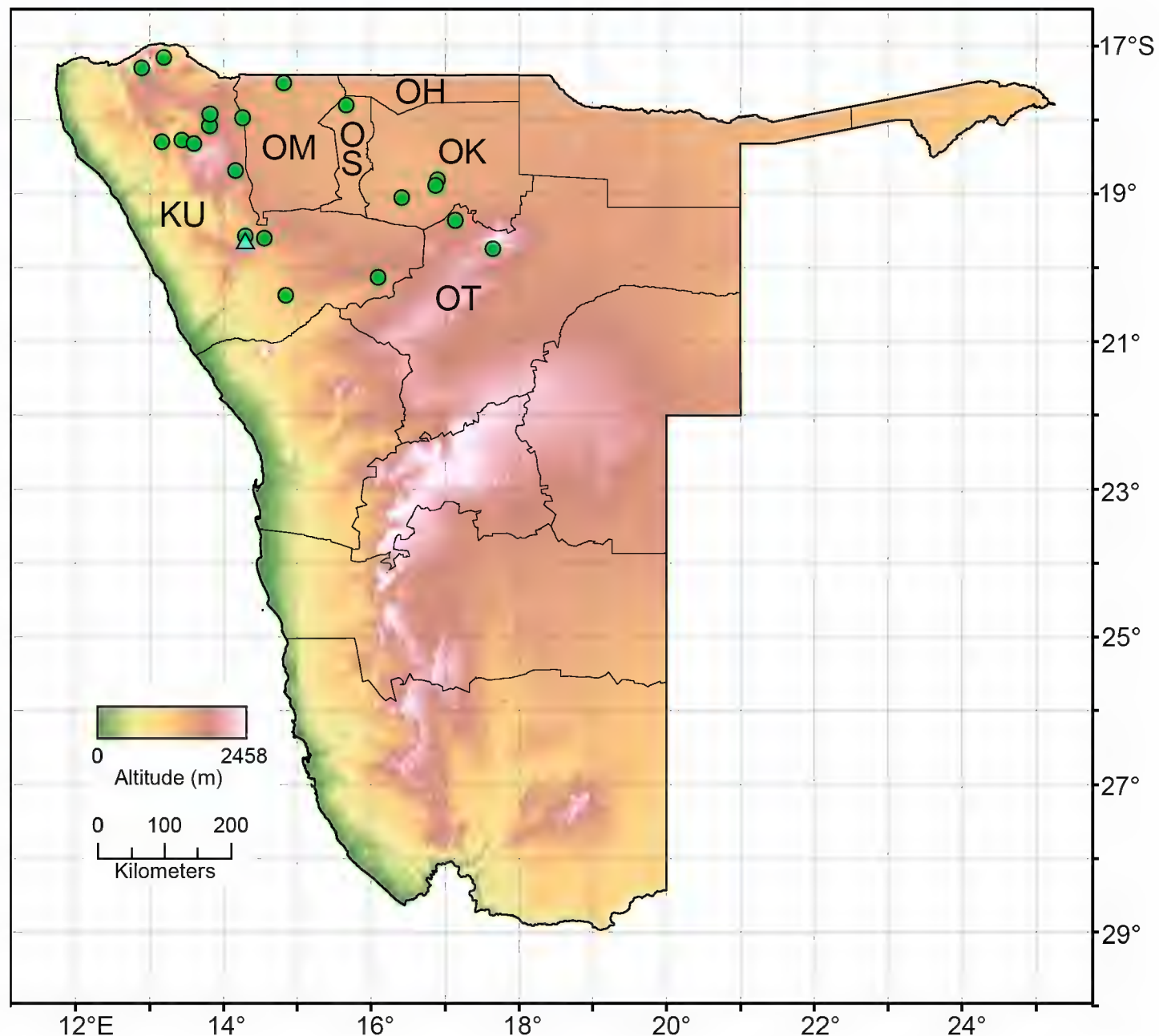


Fig. 5. Map of Namibia showing the distribution of *Nucras damarana* (green circles) and *N. cf. broadleyi* (blue triangle). Black borders indicate regional boundaries. Regions mentioned in the text are: KU – Kunene, OH – Oshana, OM – Omasati, OS – Oshana, OK – Oshikoto, and OT – Otjozondjupa. Map courtesy of Edward L. Stanley.

as woodland, but extending into areas of sparse shrubland (Atlas of Namibia 2002). *Nucras damarana* is an uncommonly encountered terrestrial species, usually found in relatively mesic microhabitats in areas with at least some vegetation as ground cover. Although the diet has not been studied in this species, most congeners have a broad diet of arthropods including various insects, spiders, and centipedes (van der Meer et al. 2010), and this is likely the case for *N. damarana*.

Conservation. Although this species, like many *Nucras*, is not commonly encountered, it has a large extent of occurrence (> 80,000 km²) and its entire range falls within an area of relatively low human density. In Ovamboland, localized agricultural activity may be a threat to this terrestrial lizard, but it likely experiences minimal disruption in other portions of its range, particularly in the Kunene Region. It is protected in Etosha National Park as well as several communal conservancies and is of Least Concern.

Nucras aff. broadleyi. CAS 214642, from 48 km west of Kamanjab on the road to Torra Bay (-19.65389,

14.35083), in the Kunene Region, Namibia (Fig. 5), was strongly supported as the sister to the two Angolan samples sequenced and identified as *Nucras broadleyi* by Branch et al. (2019a). There was a 15.03–15.69% divergence in the 16S sequence between the Angolan and Namibian specimens, compared to a 7.83% divergence between the two Angolan samples. While these divergences are relatively high, they are likely artificially inflated because of ambiguities in base calls and alignment in part of the sequences obtained. When compared with the diagnostic features proposed for *N. broadleyi*, our specimen is consistent with respect to scale characters, most notably the presence of granules between the supraoculars and supraciliaries, the well-defined occipital scale separating the parietals posteriorly, and the absence of a distinct parietal window in the interparietal scale (Fig. 6C). It is also consistent in size (58.2 mm SVL vs. a maximum of 63 mm) and falls within the range of all standard but non-diagnostic features of scalation presented by Branch et al. (2019a) for *N. broadleyi*. However, the specimen differs significantly in color pattern. Branch et al. (2019a) describe the diagnostic dorsal pattern as having a series of longitudinal pale stripes, including four pale stripes

on the nape with the lateralmost of these forming a light stripe that is continuous with the outer edges of the parietals. In contrast, the Namibian specimen has a pattern entirely lacking solid longitudinal stripes and instead exhibits pale spots or dashes anteriorly and a series of irregular transverse bars posteriorly (Figs. 6–7). This most closely resembles the pattern seen in adult specimens of *N. intertexta*, which was, indeed, the field identification given to the specimen by the senior author. However, Namibian *N. intertexta* are much larger (up to 91 mm SVL) and have a larger number of dorsal midbody scale rows (40–56 vs. 38–48 in Angolan *N. broadleyi* and 38 in CAS 214642) and usually have the parietals in contact posteriorly (comparative data from Broadley 1972 and Branch et al. 2019a). The mismatch in color is noteworthy, however, both *N. intertexta* and *N. broadleyi* show significant variation in their patterns (Branch et al. 2019a: Fig. 6; Broadley 1972: Pl. III), with elements of the pattern of *N. intertexta* present even in a paratype of *N. broadleyi* (PEM R24157, Branch et al. 2019a). The recognition of species-specific patterns has confounded *Nucras* systematics since the time of Boulenger (1917, 1920). A possible, though purely speculative, interpretation could be that CAS 214642 represents mitochondrial introgression of *N. broadleyi* into *N. intertexta* in northern Namibia. Nuclear data for the Namibian sample would be needed to test this hypothesis. Alternatively, it might represent a new species allied to *N. broadleyi* or it could be conspecific with Angolan *N. broadleyi*. Under the last interpretation one would have to assume both that color pattern is very highly variable and that more (and more complete) sequence data would likely reveal less pronounced genetic distances between samples. If conspecific, this would represent a substantial range extension southward and would expand the extent of occurrence of *N. broadleyi* to over 117,000 km². Based

on the present evidence, given that we have only a single Namibian sample and very limited DNA sequence data, we refer this single sample to *N. aff. broadleyi*.

Discussion

The pattern of relationships retrieved here for *Nucras* was similar to other recent molecular results published by Edwards et al. (2013), Bauer et al. (2019), and Branch et al. (2019a). However, the recently described *N. broadleyi* was recovered as the sister species to the East African *N. boulengeri*, rather than as sister to the *N. tessellata*/*N. lalandii* clade (Branch et al. 2019a). All patterns of relationship within the main southern African clades, the *N. tessellata*/*N. lalandii* clade, and the *N. ornata*/*N. holubi* clade, are fully consistent with earlier findings, with the exception of the placement of *N. taeniolata*. This taxon, endemic to the Eastern Cape Province of South Africa, was previously recovered as the sister to *N. tessellata*, although with very shallow branch lengths subtending the species pair (Branch et al. 2019a; Edwards et al. 2013). Here we recover *N. taeniolata* embedded deeply within *N. tessellata*. Although two of our genetic markers (16S and ND4) overlap with these other studies and many of the same samples were used (see Table 1), our differing results may nonetheless reflect the differences in either genetic marker choice or taxon sampling, or both. The difference in the placement of *N. broadleyi* relative to Branch et al. (2019a) is likely due to the absence of any nuclear markers in our dataset.

The finding that *Nucras holubi* as currently construed is a species complex is novel, although results from Branch et al. (2019a) did show a deep divergence within the species. That the Namibian populations, here resurrected as *N. damarana*, should be specifically distinct is not surprising, given the large geographic disjunction from *N. holubi* in southeastern Africa. The



Fig. 6. *Nucras* aff. *broadleyi* (CAS 214642). (A) Whole body dorsum, (B) lateral view of head, and (C) dorsal view of head. Note the patterning resembling *Nucras intertexta* and the prominent occipital scale. Photos by M. Murdoch.



Fig. 7. Life photo of *Nucras* aff. *broadleyi* (CAS 214642) from 48 km west of Kamanjab on the road to Torra Bay (-19.65389, 14.35083), Kunene Region, Namibia. Photo by Randall Babb.

finding of two deeply divergent lineages of *N. holubi* in South Africa is unexpected. The two clades correspond to a clade north of the Soutpansberg and sister to *N. damarana*, and another clade represented by specimens from Limpopo Province south of the Soutpansberg plus a specimen from KwaZulu-Natal, at the extreme southeast of the species range (Burger 2014). Without much more extensive sampling, the ranges of these two lineages of *N. holubi* remain uncertain. The type locality given by Steindachner (1882) is “aus dem Thale des Krokodilflusses in Transvaal.” Based on Emil Holub’s travels (Holub 1881: 83), this would have been somewhere between the confluence of the Marico and Crocodile rivers and the junction of the Notwane River with the Limpopo River along what is today the Botswanan border with western Limpopo Province, South Africa. This location is well to the west of any of our samples and warrants further investigation, including toponymic genetic sampling and a careful morphological comparison of the types with specimens from throughout the range. In resurrecting *N. damarana*, we were able to identify several features that distinguish it from all *N. holubi sensu stricto*, but we did not attempt to distinguish among the latter.

The identification of a *Nucras* allied to *Nucras broadleyi* in northern Namibia was also surprising. The specimen was field identified as *N. intertexta*,

but was placed outside of all other southern African *Nucras* in preliminary analyses. The inclusion of *N. broadleyi* into our data set provided clear evidence that our specimen is most closely related to *N. broadleyi*, but there is a substantial difference in color pattern as well as a large genetic distance between our Namibian specimen and those reported by Branch et al. (2019a).

With the addition of our new data there are now four *Nucras* species recognized in Namibia: *N. tessellata* (widespread south of 22°N, except in the Namib), *N. intertexta* (widespread in central and western Namibia north of Windhoek, except in the Namib), *N. damarana* (endemic to northwestern Namibia), and *N. aff. broadleyi* (a single locality in the southern Kunene Region). Our single *N. aff. broadleyi* was found essentially in sympatry with *N. damarana* on the Kamanjab Plateau, where *N. intertexta* also occurs. Records of *N. intertexta* from northern Namibian localities should be reexamined given that our Namibian *N. broadleyi* demonstrates that specimens phenotypically similar to *N. intertexta* may, in fact, carry *N. broadleyi* DNA. Our record lies approximately 275 km south of the reported range of *N. broadleyi* (Branch et al. 2019a). While substantial, this alone should not rule out some connectedness of the Angolan and Namibian populations, given that

Nucras are relatively infrequently encountered and that the intervening region remains poorly explored herpetologically. A similar situation exists in the skink *Trachylepis laevis*, described from Angola (Boulenger 1907) and many decades later found as far south as the Kamanjab area (Bauer et al. 1993; Steyn and Mitchell 1965); and also in *Tomopterna ahli*, which was described as *T. damarensis* from Khorixas, southern Kunene Region and 15 years later was revealed to be as widespread as 538 km to the northwest in Namibe Province, Angola. Indeed, southwestern Angola shares a high herpetofaunal similarity with northwestern Namibia, and the Kunene Region in particular (Branch et al. 2019b; Marques et al. 2018).

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Literature Cited

Arèvalo E, Davis SK, Sites Jr JW. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology* 43: 387–418.

Atlas of Namibia Project. 2002. Atlas of Namibia. Directorate of Environmental Affairs, Ministry of Environment and Tourism, Windhoek, Namibia. Available: http://209.88.21.36/Atlas/Atlas_web.htm [Accessed: 2 June 2020].

Bates MF. 1996. New reptile distribution records for the Free State Province of South Africa. *Navorsinge van die Nasionale Museum Bloemfontein* 12: 1–47.

Bauer AM, Branch WR, Haacke WD. 1993. The herpetofauna of the Kamanjab area and adjacent Damaraland, Namibia. *Madoqua* 18: 117–145.

Bauer AM, Childers JL, Broeckhoven C, Mouton

PLFN. 2019. A new *Nucras* Gray, 1838 (Squamata: Lacertidae) from the Strandveld of the Western Cape, South Africa. *Zootaxa* 4650: 149–163.

Boettger O. 1893. *Katalog der Reptilien-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main. I. Teil (Rhynchocephalen, Schildkröten, Krokodile, Eidechsen, Chamäleons)*. Gebrüder Knauer, Frankfurt am Main, Germany. x + 140 p.

Boettger O. 1894. Aufzählung der Arten [in Dr. Eduard Fleck's Reiseausbeute aus Südwest-Afrika. II. Vorkommen und Lebensweise der Reptilien und Batrachier, p. 83–93]. *Bericht über die Senckenbergische naturforschende Gesellschaft in Frankfurt am Main* 1894: 88–93.

Borowiec ML. 2016. AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ* 4: e1660.

Boulenger GA. 1887. *Catalogue of the Lizards in the British Museum (Natural History). III. Lacertidae, Gerrhosauridae, Scincidae, Anelytropidae, Dibamidae, Chamaeleontidae*. Trustees of the British Museum, London, United Kingdom. xii + 575 p., pl. I–XL.

Boulenger GA. 1910. A revised list of the South African reptiles and batrachians, with synoptic tables, special reference of the specimens in the South African Museum, and descriptions of new species. *Annals of the South African Museum* 5: 455–538.

Boulenger GA. 1917. A revision of the lizards of the genus *Nucras*, Gray. *Annals of the South African Museum* 13: 195–216, pl. VI–VII.

Boulenger GA. 1920. *Monograph of the Lacertidae, Volume 1*. British Museum of Natural History, London, United Kingdom. x + 352 p.

Bourquin O. 2004. Reptiles (Reptilia) in KwaZulu-Natal: 1. Diversity and distribution. *Durban Museum Novitates* 29: 57–103.

Branch WR. 1998. *Field Guide to the Snakes and other Reptiles of Southern Africa*. Struik Publishers, Cape Town, South Africa. 399 p.

Branch WR, Conradie W, Vaz Pinto P, Tolley KA. 2019a. Another Angolan Namib endemic species: a new *Nucras* Gray, 1838 (Squamata: Lacertidae) from south-western Angola. *Amphibian & Reptile Conservation* 13(2) [Special Section]: 82–95 (e199).

Branch WR, Vaz Pinto P, Baptista N, Conradie W. 2019b. The reptiles of Angola: history, diversity, endemism, and hotspots. Pp. 283–334 In: *Biodiversity of Angola. Science and Conservation: a Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.

Broadley DG. 1965. Some problems presented by the sand lizards of the *Nucras tessellata* group. *Journal of the Herpetological Association of Africa* 1: 18–23.

Broadley DG. 1972. A review of the *Nucras tessellata* group (Sauria: Lacertidae). *Arnoldia (Rhodesia)* 5(20): 1–35.

- Burger M. 2014. *Nucras holubi* (Steindachner, 1882) Holub's Sandveld Lizard. Pp. 167 In: *Atlas and Red List of the Reptiles of South Africa, Lesotho, and Swaziland*. Suricata 1. Editors, Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, de Villiers MS. South African National Biodiversity Institute, Pretoria, South Africa. 485 p.
- De Waal SWP. 1978. The Squamata (Reptilia) of the Orange Free State, South Africa. *Memoirs van die Nasionale Museum* 11: i–iii + 1–160.
- Edwards S, Tolley KA, Vanhooydonck B, Measey GJ, Herrel A. 2013. Is dietary niche breadth linked to morphology and performance in Sandveld lizards *Nucras* (Sauria: Lacertidae)? *Biological Journal of the Linnean Society* 110: 674–688.
- Fischer JG. 1888. Über eine Kollektion Reptilien von Angra Pequenna. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten* 5: 11–17.
- FitzSimons VM. 1943. The lizards of South Africa. *Memoirs of the Transvaal Museum* 1: i–xv + 1–528 p., 24 pl., 1 folding map.
- Gouldie A, Viles H. 2015. *Landscapes and Landforms of Namibia*. Springer, Dordrecht, Netherlands. xi + 173 p.
- Griffin M. 2003. *Annotated Checklist and Provisional National Conservation Status of Namibian Reptiles. Biodiversity Inventory*. Namibia Scientific Society, Windhoek, Namibia. [2] + 169 p.
- Heinicke MP, Ceríaco LMP, Moore IM, Bauer AM, Blackburn DC. 2017. *Tomopterna damarensis* (Anura: Pyxicephalidae) is broadly distributed in Namibia and Angola. *Salamandra* 53: 461–465.
- Herrmann H-H, Branch WR. 2013. Fifty years of herpetological research in the Namib Desert and Namibia with an updated and annotated species checklist. *Journal of Arid Environments* 93: 94–115.
- Hewitt J. 1910. A key to the South African species of Geckonidae, Scincidae, Gerrhosauridae, and Lacertidae, together with some notes on the specific characters and a brief summary of the known facts of their distribution. *Annals of the Transvaal Museum* 2: 77–115.
- Hoesch W, Niethammer G. 1940. Die Vogelwelt Deutsch-Südwestafrikas namentlich des Damara- und Namalandes. *Journal für Ornithologie* 88 (Sonderheft): i–v + 1–404 p., 8 pl.
- Holub E. 1881. *Sieben Jahre in Süd-Afrika. Erlebnisse, Forschungen und Jagden auf meinen Reisen von den Diamantenfeldern zum Zambesi (1872–1879). Band 1*. Alfred Hölder, Wien, Austria. xvi + 528 p., 1 folding map.
- Jacobsen NHG. 1989. A herpetological survey of the Transvaal. Ph.D. Dissertation, University of Natal, Pietermaritzburg, South Africa. 1,618 p.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.
- Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ. 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* 14: 91–104.
- Marques MP, Ceríaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and distribution of the amphibians and terrestrial reptiles of Angola: atlas of historical and bibliographic records (1840–2017). *Proceedings of the California Academy of Sciences* 65(Supplement II): 1–501.
- Mertens R. 1955. Die Amphibien und Reptilien Südwestafrikas. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 490: 1–172.
- Mertens R. 1971. Die Herpetofauna Südwest-Afrikas. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 529: 1–110.
- Methuen PA, Hewitt J. 1914. Records and descriptions of the reptiles and batrachians of the collection [The Percy Sladen Memorial Expedition to Great Namaqualand, 1912–1913: Zoology]. *Annals of the Transvaal Museum* 4: 118–145, pl. 14.
- Miller M, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. IEEE, New Orleans, Louisiana, USA.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The Simple Fool's Guide to PCR, Version 2.0*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii at Manoa, Honolulu, Hawaii, USA. 45 p.
- Parker WW. 1936. Dr. Karl Jordan's expedition to South-West Africa and Angola: herpetological collections. *Novitates Zoologicae* 40: 115–146.
- Rambaut A, Suchard M, Xie D, Drummond A. 2014. Tracer v1.6. Available: <http://beast.bio.ed.ac.uk/Tracer> [Accessed: 2 June 2020].
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Steindachner F. 1882. Über eine neue *Eremias*-Art aus dem Thale des Krokodilflusses in Transvaal. *Sitzungsberichte der mathematisch-naturwissenschaftlichen Classe der kaiserlichen Akademie der Wissenschaften, Wien* 86: 83–85, 1 pl.
- Sternfeld R. 1911a. Die Reptilien (außer den Schlangen) und Amphibien von Deutsch-Südwestafrika. *Fauna der Deutschen Kolonien* 4(2): 1–65, 1 folding map.
- Sternfeld R. 1911b. Zur Herpetologie Südwestafrikas.

Mitteilung aus dem Zoologischen Museum in Berlin 5: 393–411.

Steyn W, Mitchell AJL. 1965. A new scincid genus, and a new record from South West Africa. *Cimbebasia* 12: 2–12.

van der Meer MH, Whiting MJ, Branch WR. 2010. Ecology of southern African sandveld lizards (Lacertidae, *Nucras*). *Copeia* 2010: 568–577.

Visser J. 1984. Akkedis-familie van die Ou Wêreld. *Landbouweekblad* 2 November 1984: 64–65, 67, 69.

Werner F. 1910. Reptilia et Amphibia. In: Zoologische und anthropologische Ergebnisse

einer Forschungsreise im westlichen und zentralen Südafrika. Band IV, Systematik und Tiergeographie Vertebrata B. Editor, Schultze L. *Denkschriften der medizinisch-naturwissenschaftlichen Gesellschaft zu Jena* 16: 279–370, pl. VI–XI.

Werner F. 1915. Reptilia und Amphibia. Pp. 323–376, pl. 7 In: *Beiträge zur Kenntnis der Land- und Süßwasserfauna Deutsch-Südwestafrikas. Ergebnisse der Hamburger deutsch-südwestafrikanischen Studienreise 1911*. Editor, Michaelsen W. L. Friedrichsen, Hamburg, Germany.



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Joe Mitchell – An Unfinished Life

Craig Hassapakis and the ARC team

Amphibian & Reptile Conservation, (amphibian-reptile-conservation.org), 3709 West Lilac Heights Drive, South Jordan, Utah 84095-5100, USA

Abstract.—Personal contributions on the life and career of Joseph Calvin Mitchell (1948–2019) by family members and colleagues: Susan C. Walls, Susan Johnson, Jill A. Wicknick, Valorie Titus, Carola A. Haas, and Kurt Buhlmann.

Keywords. Influence, contributions, farewell, history, North American herpetology, researcher, turtles, Virginia

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I met Joe when the idea for creating a herpetology journal devoted exclusively to conservation was just being born, back in 1995. Joe was very supportive of the idea and lent his prestigious name to our fledgling journal as a member of the advisory board. I still remember standing in a circle of professors at a conference a quarter-century ago (1995), early in the development of the journal, and their excitement that ARC was to be a full-color scientific journal, since such a “luxury” was exceedingly rare in those days. Joe’s paper was the first scientific paper published in the journal:

Mitchell JC, Wicknick JA, Anthony CD. 1995. Effects of timber harvesting practices on peaks of Otter Salamander (*Plethodon hubrichti*) populations. *Amphibian & Reptile Conservation* 1(1): 15–19 (e3).

His submission of this article to ARC attests to Joe’s willingness to help and risk his reputation by going with a new journal with lofty ideals.

Joe continued to lend advice and review papers for ARC over the years as the journal continued to grow in prominence. One feature of Joe’s character that I could always count on in my collaborations with him through the journal was that no matter how busy his schedule was, Joe always came through with very insightful and helpful manuscript reviews, which always impressed me and spoke to his commitment to conservation and the journal as well.

An example of one my may last communications with Joe, which neatly summarizes his willingness to help and the many responsibilities which must invariably come with such openness, is his response to a manuscript I had just sent him to review which was packed full of behavioral field observation data:

Monday, February 11, 2019 4:45 PM

OK, Craig. I downloaded them all in a folder. Data-rich is an understatement. I’ll get to it as soon as I can. Lots on my plate.
Joe

It was commented to the editorial team that Joe was “one of our best reviewers for American reptiles and amphibians!”

Joe left this world all too soon and he is sorely missed by all who knew him. The entire ARC team wants to thank Joe for all his dedication and help in getting the journal *Amphibian & Reptile Conservation* started and helping us to improve the submitted manuscripts over more than two decades. We are forever grateful and know that Joe’s spirit will continue on into the future as he was an inspiration to all of us. While Joe’s contributions to natural history and herpetology have been reviewed in previous memorials, several of his family members and colleagues have submitted some more personal thoughts on their memories of knowing and working with Joe over the years.

Susan C. Walls (Joe's wife)

Fort White, Florida, USA

Tuesday, 2 July 2019, began like any other day for Joe Mitchell. I left home early that morning, before he awakened, to attend an out-of-town meeting. When I got home in the late afternoon, though, it was easy to reconstruct how his morning had unfolded. He had cooked sausage for breakfast, and only had half a cup of coffee—the other half would be waiting for him on the kitchen counter when he got home. He only sent one email that morning, and it was about an undergraduate he was mentoring—clearly something that was important to him since he tended to that before doing anything else. He then loaded the bed of his pickup truck with our recycling bins and made a run to the local recycling center to drop it all off. On his return trip, he had planned to stop at the local bank to deposit a check—the check and a deposit slip were still on the passenger seat of his truck when I retrieved it the next day from the tow yard. At some point before leaving, he snapped a couple of pictures on his cell phone of two of our dogs, curled up in the new “fox hole” they had just dug in the yard. He most likely was amused by their creative digging and he would have chuckled as he shared the pictures with me later that night when I got home. After getting the dogs back in the house he left for the recycling center, only “dummy locking” the gate—something we did out of laziness when we knew we were just stepping out for a moment. He obviously didn't plan to be gone long and was probably already thinking ahead to the next item on his to-do list for the day. But Joe never made it back from

the recycling center; something blew out of the bed of his pickup on his return trip home. He pulled over and darted out into the highway to get it. And, in the blink of an eye, he was gone. The driver of a semi-truck approaching from behind swerved at the last minute but couldn't avoid impact. What started out as just an ordinary day ended in tragedy, loss, and grief for Joe's family and friends, the herpetological community, and me.

The herpetological community knew Joe for his stature within, and contributions to, the field of herpetology. Joe was a loyal member of The Herpetologists' League, The Society for the Study of Amphibians and Reptiles, The American Society of Ichthyologists and Herpetologists, and the Virginia Herpetological Society. He was our esteemed colleague, a herpetologist extraordinaire, and a gentle soul. But he was so much more. He was a loving and devoted father and grandfather, brother, uncle, cousin, brother-in-law, and husband. His family meant the world to him, as did his colleagues, whom he once referred to as his “tribe.” Joe was also a Marine—as he said frequently, “once a Marine, always a Marine.” He was very proud of his military service during the height of the Vietnam War. If he ever saw another Marine veteran when we were out, he greeted them with “Semper Fi, brother. Semper Fi.”

Joe was also a mentor to many young people, past and present. Over the last several years he had thoroughly enjoyed working with Jerry Johnston of the Santa Fe College in Gainesville, Florida, and his students working on turtles in the Santa Fe River system near our home in Columbia County, Florida (Fig. 1). Jerry tells me that Joe was actively mentoring many of them, and I am so glad that these young people had the opportunity to get to



Fig. 1. Joe showing Santa Fe College students how to count growth rings in *Pseudemys concinna* as part of a long-term demographic study of river turtles.



Fig. 2. Joe as a high school senior with his award-winning pair of mahogany lamps and a pet Mole King Snake, *Lampropeltis calligaster*.

know him and learn from him.

Joe had many interests outside of herpetology as well. Since high school he had a strong interest in wood-working. In his high school senior year, he made a pair of mahogany lamps (among other pieces of furniture; Fig. 2). Their intricate and unique spiral design took seven weeks to finish and involved lots of hand sanding and finishing, but his efforts won him first place in the senior wood turning class at the county and state industrial arts shows. The summer after he graduated from high school, he apprenticed with a furniture maker and intended to

pursue that career path but ended up joining the U.S. Marine Corps on his 18th birthday in August, 1966. In the recent few years before his death, Joe was starting to get back into wood-working again in his home shop.

Joe was a true bibliophile. His expansive herpetological library (Fig. 3) was admired by many, but his literary interests were not limited to just herpetology. In addition to his herpetological book, journal, and reprint collections, Joe collected books on natural history, military history, leadership, and Native American culture and philosophy. Joe's allegiance to the Marines fueled his interest in military books and, from a young age, he had been intrigued by Native American history and culture. Joe was not a religious person but he was very spiritual, and he aligned himself with many Native American beliefs and philosophies. In his later years, Joe developed a strong interest in leadership, and his library holdings indicated that he studied a variety of leadership approaches. In addition to books, Joe collected high-end custom-made knives and baseball caps (especially those bearing Marine Corps emblems), and he was passionate about bluegrass music—the more energetic the banjo, the better! Joe once took banjo and voice lessons with the hopes that he could play bluegrass himself, but he never became proficient enough to do so.

Joe described himself as being “product-oriented” and he was a prolific writer. He also thoroughly enjoyed the editorial process. At the time of his death, he was co-editing *Snakes of Arizona* (with Andy Holycross)



Fig. 3. A portion of Joe's herpetological book collection, housed in barrister bookcases willed to Joe from Roger Conant. The bookcases originally belonged to Roger's father.



Fig. 4. Joe at work at his desk with his best canine friend, Jake, close by.



Fig. 5. Joe and Jake in 2011.

and he had a long list of planned book projects and research articles lying in wait (most notably a book on the herpetology of the Delmarva Peninsula with Roger Conant). At age 70, Joe was far from ready to settle into the more sedentary life one typically associates with “retirement.” When he wasn’t working, he enjoyed relaxing on his 6-acre wooded property in North Central Florida with his best canine friend Jake, a rescued stray, never far away (Figs. 4, 5).

Joe always cautioned me not to ruminate on things, so I know that he would not want us to dwell on the unspeakable tragedy of his loss. Joe cheated death ten years earlier when he had a heart attack that required bypass surgery; sadly, however, he wasn’t able to cheat death a second time. Joe had told me once that, upon his death, he wanted a party with lots of bluegrass music rather than a morose funeral. I honored his request. And, as hard as it is to do sometimes, I think that Joe would prefer that we celebrate his life rather than mourn his loss.

Semper Fi, Joe.

Susan Johnson (Joe’s sister)
Mechanicsville, Virginia, USA

It was fun, and sometimes a bit crazy, growing up with a future herpetologist...snakes and a few lizards were always part of our family. (For some reason we could not have a dog, but my parents OK’d the snakes!) At one point, Joe’s bedroom had a cot in the center and was surrounded by shelves of aquariums which contained mostly snakes and a few lizards. Our den had a large aquarium housing Joe’s Boa Constrictor while the utility

room had a shelf of preserved snakes in large jars. Yes, our family and friends thought this odd, and not just a little bit crazy. The credit for inspiring Joe’s passion for herpetology goes to our Uncle Cos, and kudos to our parents for allowing Joe to follow that passion at a young age. I really cherish the experience of handling and living with snakes and other critters as I grew up. Joe taught me to not fear them but to be in awe of them and the rest of our natural world.

Jill A. Wicknick

University of Montevallo, Montevallo, Alabama, USA

A solid man, not tall, stood at the back of his pickup. The Semper Fi sticker gave a hint at the physical exertion of the upcoming field work. With a flash of his broad, welcoming smile, Joe Mitchell was ready to get started.

The 16 km segment of the Blue Ridge Parkway where *Plethodon hubrichti*, the Peaks of Otter Salamander, has resided for five million years was my home for three autumn seasons of field research on competition and territoriality. Living in a 17’ travel trailer in a campground that was devoid of other humans on weekdays, I shared the area with a young black bear, a bobcat, and a park ranger who was either a poor shot or didn’t have the heart to kill a downed deer. This was the setting where I met Joe Mitchell in his territory: Virginia. He was happy to show it off, happy to mentor a graduate student, happy to be in the field; ear-to-ear-grin, whole-face-alight happy.

Joe and I had already met at the herp meetings, but this was our first time in the field together. We were in a high-elevation valley nestled between two nearby peaks: Sharp Top Mountain at 3,875 ft, and Flat Top Mountain at 4,004 ft. I was just starting my dissertation project and



Fig. 6. Joe's presentation at an NEPARC meeting on the reptiles and amphibians of Virginia's Civil War sites.

Joe had experience with the localities for my study. He also knew that the area contained high quality timber and was concerned about the effects of timbering on this vulnerable endemic. He helped me to get my project started, and he invited me to work with him examining the effects of timber harvesting on *P. hubrichti*. In the process, he nurtured my thinking about conservation.

While we worked at the Peaks of Otter, Joe showed me how to select research sites on a map and how to ground-truth them. Machete in control but flying, he created transects and taught me field methods. I think of him when I look at my own machete which hangs in my office.

Joe appears on my CV eight times, mostly from 1994–1997 and all related to *Plethodon hubrichti*. Our timbering publication, with C.D. Anthony, appeared in the inaugural issue of ARC which Joe was thrilled to be a part of—enthusiastic about the new journal's conservation focus and eager to publish in it. He talked a lot about publishing. He wanted to leave a herpetological legacy through his publications but he has left so much more. In addition to his manuscripts, he had a lifetime of teaching others while sharing his enthusiasm, his friendship, and his zest for life. The knowledge he passed on still continues its journey.

Valorie Titus

Keystone College, LaPlume, Pennsylvania, USA

Though it's been over a year, it is still hard to sit down and write this. I first met Joe in 2003 at a Northeast Partners in Amphibian and Reptile Conservation

(NEPARC) meeting in West Virginia. I was a very green grad student, in the middle of my Master's degree work on copperheads. He seemed quite amused by this young female Yankee trying to figure out the behavior of these snakes in Kentucky, and he was a very engaging and supportive person from the start. (He later encouraged me to submit the story of my first copperhead encounter for publication, but that's a story for another day!)

My next encounter with Joe was at the JMIH in Norman, Oklahoma in 2004. This was my first big meeting and my first ever presentation. He was so excited to introduce me to many of the other herpetologists and was just a networking encyclopedia. Since then, I had always looked forward to seeing Joe at conferences and meetings. My favorite ones were always the NEPARC and SEPARC meetings, where we could take the time to catch up over a good beverage. One NEPARC meeting in Virginia particularly stands out. Joe was our keynote speaker (Fig. 6), and he came in dressed like quite the southern gentleman and talked (in a very pronounced southern drawl) about the work he had done on various Civil War sites (while joking that Virginia should be considered a Southern state). He was always a joy to watch when he made presentations.

Joe was always ready for a good story or to offer some sage advice or encouragement. I knew that I could always send him an email with a question and get a quick reply. He was really integral in offering me support whenever I was struggling with a chapter in my dissertation or a publication. He always encouraged me to just keep on plugging away and keep up my enthusiasm. If it wasn't for Joe's influence, I would never have become so involved with PARC--and for that alone, I am exceedingly grateful.

Now, at this point in my career, while I am not much of a publishing academic, I have taken what I learned from Joe and my other mentors, and am applying it to teaching the next generation of undergraduates in the field of wildlife studies. I often try to think about what my own fears and trials were like—and what advice Joe would have given me if I were in the position of my students today. It gives me comfort knowing that his legacy will live on through me as an educator and mentor. Rest easy, Dr. Mitchell.

Carola A. Haas

Virginia Tech, Blacksburg, Virginia, USA

I first met Joe Mitchell in 1993, the year I arrived at Virginia Tech. I can no longer remember the exact circumstances, but I think Joe was in Blacksburg to meet with someone else at the university or to meet with Sue Bruenderman, who at that time was the Non-Game Aquatics coordinator for the Virginia Department of Game and Inland Fisheries (VDGIF). Someone must have thought it would be a good idea to connect Joe with a new faculty member who had an interest in amphibians, so I think Joe looked me up or stopped by, and somehow, we were introduced. I know that by then I'd heard his name several times as someone I needed to connect with.

I had originally been hired by Virginia Tech to work on non-game songbirds, but having landed in the southern Appalachians, I was eager to expand my research to include plethodontid salamanders, and an invitation to participate in a silvicultural experiment by colleagues in our Forestry Department had gotten me started on such a project. It quickly became clear that there was a greater need (and more funds) for research on the conservation and management of amphibians and reptiles in Virginia than for songbird work, and Joe was one of the few people currently filling that niche. At the time he was an adjunct faculty member at University of Richmond, teaching Biology courses at night and doing independent research and consulting work during the day.

Joe had been involved in the early studies of bog turtles in southwest Virginia, and was very concerned that conservation efforts for this species in the southeast needed to increase. Based on discussions he had with Kurt Buhlmann, he worked with Sue Bruenderman (VDGIF) and Alison Haskell (USFWS) to negotiate support for research on bog turtle movement and the importance of streams as movement corridors between isolated wetlands. I'm not sure how he managed to navigate the process of obtaining the always scarce "Section 6" funds before the species was listed, but Joe was often able to find ways to get folks to address work that he saw as urgent. The logistics of travelling from Richmond to southwestern Virginia to do this work were daunting, and so Joe approached me about collaborating on this project. I protested that I didn't know a thing about turtles, but

Joe assured me that he'd teach me whatever I needed to know about working with turtles and that my expertise on movement behavior and corridors was the perfect fit for the project. By that time Mike Pinder had replaced Sue at VDGIF, and together we embarked on the bog turtle research that I've continued on and off ever since.

Joe always welcomed me as a collaborator and colleague, and he encouraged me in my shift to herpetological work. As I was typing this just now, I happened to notice his *Reptiles of Virginia* book out on my side table, as I had been referring to it while working on a bog turtle manuscript within the last couple of weeks. I just looked to see what the inscription said, and found his prophetic words from October 1994: "maybe you are discovering that herps are just as exciting as birds" (Fig. 7). It would have been easy for Joe to try to stake out his territory, and treat me as a competitor rather than a collaborator, especially because his financial livelihood depended on continuing to get contracts for work. But Joe was consistently open and encouraging. I know he had plenty of conflicts over the years. Like most of us, he certainly wasn't immune to feeling like his toes were being stepped on, that he was being taken advantage of or snubbed, and there were plenty of people that got irritated at Joe too. But besides being passionate about the science, and the organisms, and the important work getting done, Joe cared deeply about people and was very invested in mentoring and supporting other herpetologists. Every time I spoke with him while I was

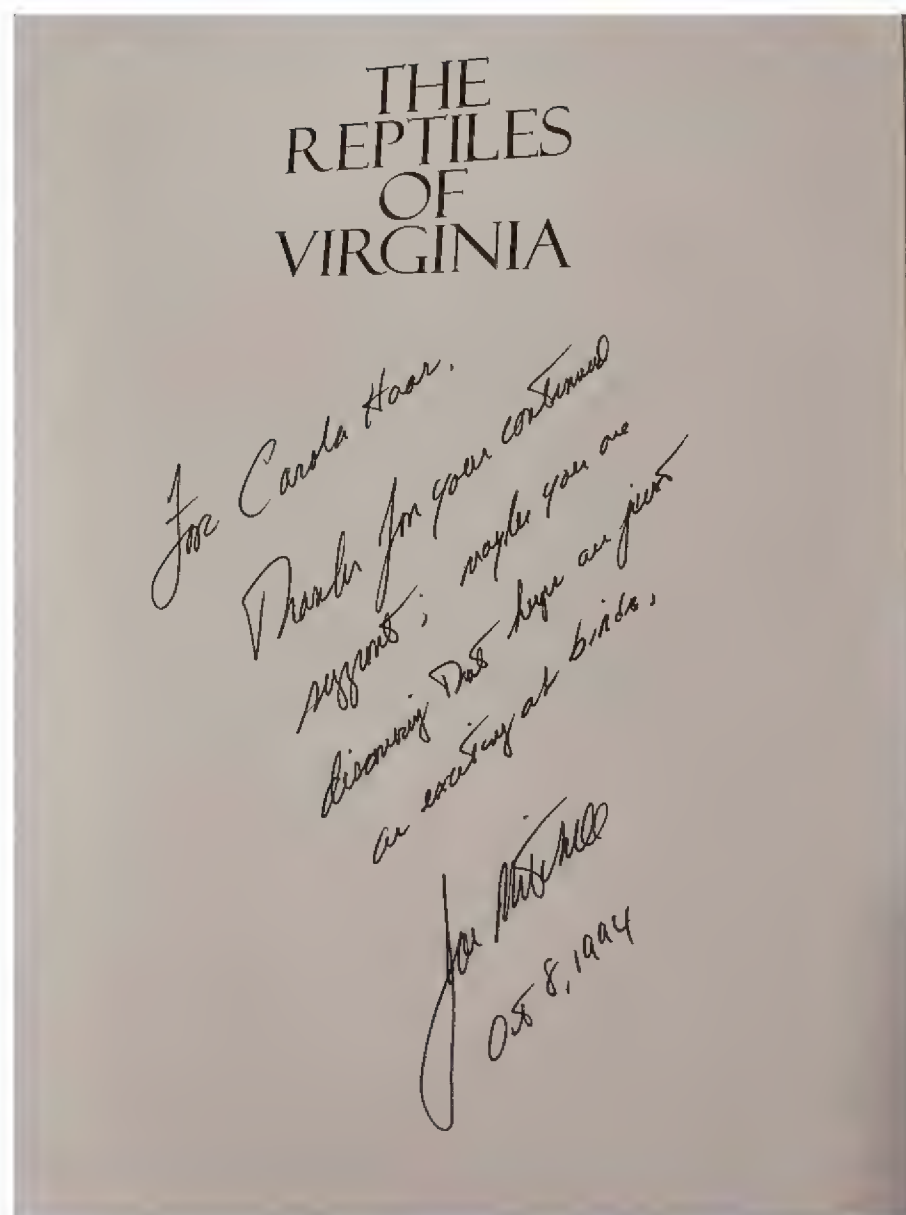


Fig. 7. Joe's inscription to me in my copy of his 1994 *Reptiles of Virginia* book.

untentured, he asked how my publications were coming along, and reminded me to make publishing my priority. He was a great mentor and source of support throughout my career.

Joe was committed to seeing work through to publication. Much of the contract work that Joe took on did not require the publication of results, but Joe was always adamant about collecting high-quality data and making sure it was available to others through peer-reviewed publications. His painstaking attention to detail, recording all the morphological and natural history information that he could, sometimes made field work with him slow. (I remember becoming hypothermic while sitting on the ground with him one February night at Maple Flats measuring all the ambystomatid salamanders that others were dipnetting!) He was dedicated to the profession in other ways as well, serving as an officer in local and national herpetological organizations, and always being willing to help with education and outreach.

I know that Joe had plenty of struggles with mental and physical health issues, many as a result of his military service. He worked hard to overcome or manage these struggles, and his willingness to acknowledge difficult circumstances and discuss his struggles helped normalize these challenges for others in the field. A driving force for Joe was always his family. His deep love for all his family members was so obvious from conversations with

him. He was a staunch ally to family and friends alike.

It was an honor and a joy to have worked with Joe, and I know that his contributions to herpetology and to conservation will live on.

Kurt Buhlmann

University of Georgia, Savannah River Ecology Laboratory, Aiken, South Carolina, USA

Some personal remembrances of a great mentor and friend, Joe Mitchell

I first heard the name, Dr. Joseph C. Mitchell, when I began my literature search as a new MS graduate student at Virginia Tech (VT, Blacksburg, Virginia), in the Fall of 1983. I was going to conduct a survey of birds, small mammals, amphibians, and reptiles in the newly designated New River Gorge National River corridor in West Virginia. I was just starting my search of the natural history literature so I could understand the distributions and state of knowledge of the herps I might encounter. I had recently finished my B.S. in Environmental Studies at Stockton State College in New Jersey (the state where I grew up), thus I was new to Virginia. Given that I was in the Fisheries and Wildlife Department at VT, it seemed likely that I should have been able to be quickly pointed in the right direction. However, in 1983, most wildlife



Fig. 8. Virginia Herpetological Society meeting, 1987.



Fig. 9. Whit, Kurt, and Joe, Aiken, South Carolina, 1995.

departments, including VT's which I loved being a part of, had almost no emphasis on herps, being focused primarily on game mammals and birds. However, one day I picked up an issue of *Virginia Wildlife* (Vol. 35, No. 4, [April] 1974) and it included an article on the Snakes of Virginia, with a fold-out color plate, written by Dr. Mitchell. After reading the article, I realized that perhaps this guy, who was associated with the University of Richmond, might be able to help me to become familiar with the herps of the Virginias. So, I wrote him a letter. Soon after, I received a fat manila envelope from Joe in the mail. It was filled with scientific reprints by him and other biologists which were related to my search for Virginia natural history knowledge.

Skipping forward a decade to the summer of 1985, while doing fieldwork in the New River Gorge, I decided that I should attend my first professional Herpetology Conference, which was a joint SSAR/HL meeting held at the University of South Florida, in Tampa. I drive down from Blacksburg in my old Chevy truck. I recall

removing the tailgate so that I would get better gas mileage, as I only had a few bucks for gas....

I met many herpetologists there for the first time, many of whom have become lifelong colleagues and friends. And I bumped into Joe for the first time. It was clear from our first discussions that both of us were excited about natural history, and in the fauna of Virginia in particular. Before the end of that SSAR/HL meeting, Joe had invited me to participate in his on-going Herp Survey project in Virginia, which would lead to the eventual publication of his *Reptiles of Virginia* (Mitchell 1994) book and many papers.

Joe took an interest in me as a young exuberant herpetologist in the making—and spent a great deal of time over the next 35 years as a mentor, colleague, and friend. Where I am going with this note is to provide a personal remembrance. My intention here is to capture memories and field trips, and to recognize Joe as the fine mentor and friend that he was. In the year since his passing, others have written tributes to Joe which



Fig. 10. Camping out in the old Shasta.

thoroughly highlight his professional accomplishments, and I cite those here for reference.

Several weeks after the 1985 Tampa meeting, Joe and I met with fellow herpetologist Chris Pague, Carnegie Museum scientists Ellen Censky and Jack McCoy, and Dr. Richard Hoffman in the southwest corner of Virginia to look for herps in the headwater streams of the Tennessee River—including the Clinch, Holston, and Powell Rivers, as well as a beautiful smaller stream named Copper Creek. It was if I had been transported to a different planet with the same basic types of animals, but the species were all new. Here, I had my first hands-on experiences with Hellbenders, Mudpuppies, Spiny Softshells, Striped Musk Turtles, Cumberland Sliders, Black Kingsnakes, and Zigzag Salamanders—all within a 3-day period! Now, I soon realized there was a downside to all this work with Joe. Joe was very much a student of museum series collections at the time, as were our Carnegie colleagues, and I was schooled in the value of series collections, but had a hard time handing over my catch knowing it was going into formaldehyde.

One of those afternoons, while turning rocks for Hellbenders in Copper Creek, I lifted a big flat rock, waited a second for the water to clear, and then my senses realized that I was looking at a beautiful greenish striped Common Map Turtle—the first I'd ever seen. My hand shot down into the water and closed around its shell, my right shoulder and the side of my face in the water. Joe was nearby and asked, "Did you see something?" I nearly hollered and lifted the little jewel to the surface, but then just froze and lowered it back to the stream bottom and stared down at it. "No, Joe, I thought I saw something, but I lost it in the current," I said. I just could not bring that turtle up—my first of the species—knowing what its fate would be. I think it was perhaps sometime in the early 2000s—about 20 years later—when I confessed this story to Joe 😊. That was also long after Joe had stopped



Fig. 11. Building silt fences for Bog Turtles in New Jersey, 2003.

making series collections himself, and although both of our pickup trucks always contained jugs of formalin; they were used only for road kills that we refused to see otherwise wasted.

After the southwest Virginia trip in summer 1985, I don't think a month passed between then and 1992 in which I was not meeting Joe somewhere in the field in Virginia to look for herps—Mt. Rogers in the Blue Ridge to find Yonalossee and Shovelnose Salamanders, winter



Fig. 12. PARC Habitat Management Guidelines course taught at Arnold Air Force Base, Tennessee, 2007. Joe in center in front of tree. *Photo by Mark Bailey.*



Fig. 13. Accepting the Paul Moler Conservation Award, for the PARC Habitat Management Guidelines (left to right: Mark Bailey, Kurt, Jeff Holmes, Paul Moler, and Joe).

visits to a pond along the New River to find breeding Jefferson's Salamanders, my introduction to the Virginia Herpetological Society and its members, looking for the Shenandoah Salamander on talus cliffs on a foggy night in Shenandoah National Park, searching for the elusive Chicken Turtles in Virginia Beach, or building drift fences at Prince William Forest Park just south of Washington, D.C. Joe gave me a set of maps for each county in Virginia so that I could accurately plot the locations of my herp finds, such as "3.2 miles South of Floyd on Rd. 8." To this day, you could drop me on any little country road in Virginia, and I can quickly figure out where I am. Joe not only taught me Virginia herps, but I learned about Virginia natural history, physiographic regions, and culture.

On a return trip to southwestern Virginia, in 1986 I think, I recall asking Joe if he could pay for my gas or mileage or something. Joe responded that the grant from the Virginia Department of Game and Inland Fisheries did not have that much, but he would pay for the food on all the field trips. That sounded like a good deal to a starving grad student. Subsequently on that same trip, I got us thrown out of an all-you-can eat buffet restaurant, and I think Joe might have regretted the work-for-food-only idea after that. We joked about that incident for the next 30 years.

I graduated from VT with my Master's in Wildlife Sciences in 1986 (with my thesis on River Cooters) and then had a few short-term wildlife jobs in Virginia, West Virginia, and New Jersey over the next year. These jobs included hacking bald eagles, radio-tracking snow-shoe hares, banding woodcock, and trapping wild turkeys, several of which were arranged by my major advisor at VT, Dr. Mike Vaughan. In early 1987 I had just written a proposal to work on Rio Grande Cooters in New Mexico with Charlie Painter, the New Mexico

State Herpetologist, and Joe called me. He thought he might get a grant from the US Forest Service to study the distribution of Cow Knob Salamanders in the George Washington National Forest—was I interested? It would be a for-hire job. Although I was sorry to turn down the project in New Mexico, the Cow Knob Salamander work with Joe would determine the trajectory of my career.

Joe and Chris Pague, Bob Glasgow, David Young, and I got to work building drift fences at high elevations in the George Washington National Forest. I recall going up there in April 1987 and promptly getting my truck stuck in snow. OK, so the salamanders were not out yet. But on 12 May 1987, we found our first one. We surveyed for salamanders for two years, and when Joe came up, we would camp out in my little Shasta trailer. I think together, Joe and I (with our academic backgrounds) learned how to interact with land management agencies and our survey work eventually resulted in the designation of the Shenandoah Mountain Crest Special Biological Area on the George Washington National Forest—a conservation victory that I know Joe was rightfully proud of.

While heading up the Cow Knob Salamander work, Joe included me on trips to the Blue Ridge Parkway to work with him on identifying Bog Turtle habitats along the parkway. We would meet at the sites, and find and mark turtles with a notching system that continues today.

When we went to Seashore State Park in Virginia Beach, Joe allowed me to have the newspaper story credit for trapping the Chicken Turtles. It was Joe's project, but he let me have the spotlight and be interviewed for our work. Joe was always happy to help promote others, while he was often willing to remain in the background. And that generosity is a unique quality.

Joe also sent me with his colleague, Richard Hoffman (another great natural historian of Virginia), out to an unusual series of sinkhole ponds where we found an



Fig. 14. Tracey, John Byrd, and Joe at SEPARC, 2010.

isolated population of Tiger Salamanders.

In the winters of 1988–1989, I worked for Joe in his sprawling steampipe distribution room in the bowels of the Biology building at the University of Richmond. One night on my way back to Richmond from visiting friends in Blacksburg, a white-tailed deer and my 1972 Chevelle had an encounter near the site of General Lee’s surrender (during the US Civil War) in Appomattox. I loaded the deer into the trunk and made frequent water stops because of my leaking radiator, but I made it to Richmond. In Joe’s basement lab, I proceeded to butcher the deer that night. Joe arrived at 7 AM the next morning to find me with a disemboweled deer all over his lab floor. Although he did say some expletive like, WTF!, he then spent the rest of the morning showing me how to properly butcher a deer. We enjoyed many stews and roasts that winter at Joe’s family home, and he helped me get a legal tag for the deer from the Game Department.

My work with Joe, and the education he provided about Virginia natural history, led me to successfully land my first “real” job with The Nature Conservancy and the Virginia Division of Natural Heritage in 1989. I know Joe had some behind-the-scenes influence which resulted in me obtaining that job, but it suited me perfectly. I knew Virginia well because of Joe, and I was eager to learn about other groups of animals too: like dragonflies, freshwater mussels, moths, and cave biota.

While working for the Natural Heritage Program, Joe and I continued to investigate the isolated, and rather weird, population of Chicken Turtles in Virginia Beach. I put radio trackers on the turtles and we learned that they leave the ponds and spend the winter buried in forested sand dunes. That work led me to contact Dr. Whit Gibbons at University of Georgia’s Savannah River Ecology Laboratory (SREL). Joe was always encouraging me

to continue my education and often pressed me about when I might go back for my Ph.D. I greatly enjoyed the Natural Heritage Program and believed strongly in its mission, but was always fascinated by those Chicken Turtles.

Joe started talking with Whit, and in 1992 Whit offered me an assistantship at the University of Georgia and the opportunity to study the Chicken Turtles and wetland conservation on the Savannah River Site. I should note here that Whit is another one of my career mentors and friends, on par with Joe. So, I left Virginia, and moved to near the Georgia/South Carolina border. However, about a year after I had begun my Ph.D. work at Georgia, Joe visited SREL. During a conversation among Whit, Joe, and myself, Whit said to Joe: “the main reason I accepted Kurt was so that you [Joe] would stop calling me and bugging me about him.” Well, apparently, I clearly owed my Ph.D. opportunity to Joe as well, and I hope he knew that I really appreciated it.

I was busy through most of the 1990s at SREL. Joe and I did not get together as often as we used to, although he came to SREL several times and volunteered his expertise training graduate students and technicians on proper preservation techniques for road-killed snakes and other herps. This was greatly appreciated and it was purely voluntary on Joe’s part, but I’m sure that some of our other SREL colleagues reading this here will recall our “herp pickling parties.” However, Joe and I did manage a road trip together in 1993 to attend a turtle meeting held in Purchase, New York. Having grown-up in New Jersey, I wanted to show Joe some ecological highlights of the state. Joe had never been there and joked that, as a Virginia native, he really was not comfortable north of the Mason-Dixon line. We detoured through the Jersey Pine Barrens, stopping to swim/soak in the tannin-



Fig. 15. Joe and Kurt in Florida 2011.

stained Batsto River, and we found some Red-bellied Turtles and looked at Pine Snake habitat—a species that was only legendary in Virginia, as neither of us had ever seen one there.

I should note here that Joe did not know how to swim. So, I spent some time teaching him—having spent my high school summers teaching swimming and lifeguarding. I think my efforts helped him gain confidence in the water—at least he knew that the lifejacket was going to keep him afloat, when he was wearing it.

In 1999, a meeting held at the Atlanta Airport established the Partners in Amphibian and Reptile Conservation (PARC). Joe and I were there. Those who are familiar with the Habitat Management Guidelines (HMG) series also recognize that Joe was pivotal to the brainstorming that went into the creation of those publications. Joe co-authored the Southeast and Northeast HMGs, as we call them, and helped to co-edit the Northwest and Southwest equivalents. Joe considered the PARC HMGs to be one of the most important herpetological conservation contributions of his career, and he said so in his Smithsonian autobiography.

In 2000, I began a position with Conservation International (CI) and my wife, Tracey Tuberville, and I moved back to Virginia. Joe came to our house with a new mailbox and post as a house-warming present. (Although we no longer live in Virginia that mailbox and post are now at our current house in South Carolina). The position with CI required that I understand the politics of international conservation. And while I struggled some, Joe was there to offer support. During a freak opportunity of obtaining 7,000 confiscated Asian turtles for conservation, a now historic assemblage of people volunteered a week of their time over Christmas 2001 to

the New Year in 2002 to process, measure, and provide supportive care to these turtles outside of Miami, Florida. Joe was there to help, after driving down from Virginia. He helped to coordinate the measuring and marking of most of those animals. That event has been widely recognized as a pivotal moment in the formation of the Turtle Survival Alliance (TSA).

Joe and I have both done some herpetological consulting work. He operated Mitchell Ecological Service, LLC and he helped me establish Buhlmann Ecological Research and Consulting, LLC. I had several projects on National Wildlife Refuges which provided opportunities to manage rare turtles and their habitats. I asked Joe to help me with those, and together we traveled back up to New Jersey to the Wallkill Refuge—and brought my old camper from the Cow Knob Salamander days. Joe helped me build silt fences to trap bog turtles and we spent time surveying the herpetofauna there for the Refuge staff. And I finally got to pay Joe for his time. In a surprise twist, I also had the opportunity to work on the Bosque del Apache Refuge in New Mexico. Joe, Whit, Tracey, Justin Congdon, and I met Charlie Painter there, and we finally got to play with New Mexico turtles and all together as a group. It was great fun.

Joe moved to Florida in the early 2000s, and with his new wife, Susan Walls, they set up home near Gainesville. I think Joe struggled some with the relocation to Florida after a lifetime career in Virginia, and getting into herpetological consulting there was a bit hard for him. Joe had a heart attack in late 2009. I went down to Florida from South Carolina to help get him home from the hospital after bypass surgery and, along with Susan, to help make his house easily traversable during his recovery. As one would expect, Joe had amassed a large

library of books and teetering files, along with a knife collection, in his home office. So, it was a hazardous place for a guy shuffling around after heart surgery, but Susan took good care of him and he recovered fully.

Several years later, Joe was enjoying working on the Santa Fe River, near Gainesville, helping colleagues Jerry Johnston and others with turtle surveys. I attended one snorkeling trip in 2017 and was looking forward to another trip when we lost Joe.

I'm actually not sure how to end this tribute. I know I can keep slipping into other adventures, although I've probably over-described enough of them and hinted at others. If it is not clear by now, it should be—Joe was a great friend, an excellent mentor, and selflessly volunteered his time and resources to help young and upcoming herpetologists, and he strove to turn them into conservation biologists.

In working on this tribute, I have been amazed by how many adventures I had with Joe—and I enjoyed every one of them. I appreciate the time he spent with me, and I'm sure there are others who feel the same way. Herpetology and herpetological conservation have lost a real champion with Joe's passing. And I hope that the investments he made in me and others help us to be able to pick up and carry the torch from where he left it...

Submitted with love and respect,

Kurt Buhlmann

Final note: I chose to make this tribute personal, and so I did not detail Joe's professional accomplishments and contributions. However, the following tributes published in the last year have addressed his global contributions to the herpetofaunal and biodiversity conservation communities.

Dodd CK Jr. 2019. Joseph Calvin Mitchell (1948–2019): natural historian, turtle enthusiast, Marine, Virginian. *Herpetological Review* 50(4): 889–893.

Hilton EJ, Bauer AM, Buhlmann KA, Dodd CK Jr. 2020. Joseph C. Mitchell (1948–2019): herpetologist and natural historian of the Old Dominion. *Copeia* 108(1): 188–194.

Mitchell JC. 2019. *Biographical Sketch and Bibliography of Joseph C. Mitchell*. Smithsonian Herpetological Information Service, No. 155. Smithsonian Institution, Washington, DC, USA. 39 p.

Roble SM. 2019. Joseph C. Mitchell (1948–2019). *Banisteria: A Journal Devoted to the Natural History of Virginia* 52: 52–73.



Fig. 16. Joe with a Suwannee Cooter, Ichnetucknee Springs, Florida, 2017. Photo by Jerry Johnston.

Walls SC, Buhlmann KA, Nickerson MA. 2020. Dedication: Joseph C. Mitchell. 16 August 1948–2 July 2019. Pp. vii–xi In: *Snakes of Arizona*. Editors, Holycross AT, Mitchell JC. ECO Publishing, Rodeo, New Mexico, USA. 837 p.

Some sources of Joe's older publications:

- *Virginia Wildlife*. Joe published many popular articles in this magazine, which can be accessed at the **Library of Virginia** website (<https://www.lva.virginia.gov/>).
- *Banisteria*. Joe took great pride in writing and publishing about the natural history of Virginia, and a trove of information about Virginia natural history, much of it written or edited by Joe, can be accessed at: <https://virginiannaturalhistorysociety.com/banisteria/banisteria.htm>.
- *Catesbeiana*. Joe was active in the Virginia Herpetological Society and published in its journal, *Catesbeiana* (<http://virginiaherpetologicalsociety.com/catesbeiana>).